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REVIEW ARTICLE

Human ochratoxin A biomarkers—From exposure to effect

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Abstract

Ochratoxin A (OTA) is a nephrotoxic mycotoxin that has received particular attention because of the toxic effects, widespread occurrence in contaminated food and feed chain, suspected causal effect on nephropathies, and, more recently, possibility of exposure by inhalation in domicile and occupational settings. Biomarkers have been used not only to ascertain the role of OTA in inducing chronic renal failure diseases, but also as a means to portray general populations' risk to the mycotoxin. Biomonitoring can thus be used to assess internal OTA exposure, with no need to recognize the main source of exposure. And so it presents undeniable advantages over the monitoring of external dose. With a just right understanding of biomarkers, it is possible to follow the trail from exposure right to effect, and so contribute both to surveillance plans and etiological studies. In recognition of the long serum half-life and the renal elimination of OTA, most of the studies present serum/plasma and/or urine analyses as markers of exposure. In this review and for each of these main matrices, a comparison over the advantages and disadvantages is offered. Although currently limited, an overview of the current knowledge on OTA biomarkers and the influential role of the individual characteristics, namely gender and age, along with season and geographical location is given. Attention is also given to the ongoing debate over the existence of OTA-DNA adducts, a biomarker of effective dose regarded as an alternative to biomarkers of internal dose. Although unspecific, OTA effect biomarkers are also reviewed.

Keywords: Assessment; biomarker; biomonitoring; exposure; blood; DNA adducts; effect; β2-microglobulin; milk; ochratoxin A; urine

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1. Introduction

Ochratoxin A (OTA) contains 7-carboxy-5-chloro-8-hydroxy-4,4-dihydro-3(*R*)-methylisocoumarin (ochratoxin α; OTα) that is linked through the 7-carboxy group to L-β-phenylalanine by a peptide bond (Van der Merwe et al., 1965; see Figure 1).

Toxic effects of this natural contaminant of vegetable products entering the feed and food chains have been extensively reviewed and much of the research can be found summarized in the outputs of various international meetings and assessments (for review see IARC, 1993; JECFA, 2001, 2008). In brief, kidneys are probably the main target organ for OTA. Given the nephrotoxic properties of OTA, several nephropathies were attributed to it in both animals, such as endemic porcine nephropathy described in the 1980s in Denmark (Krogh, 1977; Jørgensen and Petersen, 2002), and humans, such as Balkan endemic nephropathy (BEN), described in residents of the alluvial plains along the tributaries of the Danube river in Serbia, Bosnia, Croatia, Bulgaria, and Romania (Krogh et al., 1977; Pfohl-Leszkowicz et al., 2007) and more recently chronic interstitial nephropathy (CIN) described in North African countries, namely Tunisia (Grosso et al., 2003) and Egypt (Wafa et al., 1998). Possible additive or synergistic effects of multiple mycotoxins make the task of delineating its role far more complex and any long-term effects are beyond foresight (Erkekoğlu et al., 2010).

Aside from nephrotoxicity, OTA has immunosuppressive properties resulting in higher susceptibility to infections, and it is teratogenic and carcinogenic (Group 2B; IARC, 1993). Furthermore, as reviewed by Rizzo et al. (2002) and Erkekoğlu et al. (2010), OTA interferes with blood coagulation (Doerr et al., 1974; Gupta et al., 1979) and carbohydrate metabolism (Suzuki et al., 1975) and inhibits protein synthesis by competing with phenylalanine in the phenylalanyl-tRNA synthase–catalyzed reaction (Bunge et al., 1978). Although Creppy et al. (1995) also described an inhibition of OTA's nephrotoxic effects in rats by the presence of phenylalanine or OTA- and phenylalanine analogues such as aspartame, the recent study of Stoev (2010) showed the inefficiency of phenylalanine as a renal protector against the carcinogenic or toxic effects of OTA in chicks. OTA toxicity is clearly determined by its toxicokinetics, which furthermore determine the features of biomonitoring, namely regarding the type, nature, and levels of metabolites or parent compound itself present, and the type of biological specimens where these can be searched for. As reviewed by Muñoz et al. (2010), OTA is well absorbed in the gastrointestinal tract and binds to blood proteins, with considerable variations in serum half-lives across species that are apparently dependent on the affinity and extent of protein binding. Also, reabsorption of OTA from the intestine, enterohepatic recirculation (Roth et al., 1988), and reabsorption in the kidney proximal and distal tubules favor its accumulation in the organism (Dahlmann et al., 1998). In fact, in humans, OTA is rather persistent, with a serum half-life ($t_{1/2}$) of about 35 days, due to unfavorable elimination kinetics (Schlatter et al., 1996). The long biological half-life in human blood facilitates biomonitoring studies, and investigations in a number of countries have documented the presence of OTA in biological specimens, mainly blood and urine.

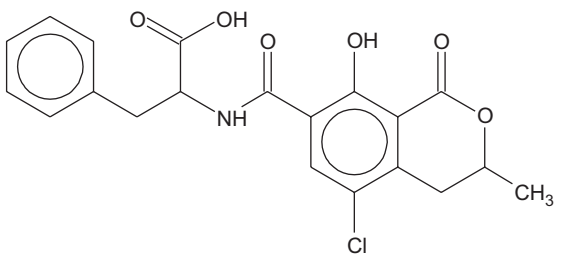

Figure 1. Chemical structure of OTA (empirical formula: $C_{20}H_{18}O_6NCl$ and molecular weight 403.82).

Biomarkers are important and essential tools, which enable the measurement of the exposure to a toxic agent and the extent of any toxic response, as well as the prediction of the likely response. Several classifications of biomarkers have already been given. The three generally accepted categories (biomarker of exposure, of response or toxic effect, and of susceptibility) may overlap sometimes, and categorization can then become confusing. Nevertheless, regardless of its class, by definition, a biomarker should be quantitative, sensitive, noninvasive, specific, easily measurable, relate to the biochemical mechanism, and work at realistic doses. The type of biomarker selected depends upon many factors, including the information that is sought, availability, and validity (Timbrell, 1998).

A suitable biomarker for OTA exposure has been sought over a number of years and various putative biomarkers have been investigated, with promising results to a correct evaluation of the risk associated with the ingestion or inhalation of these natural toxins. Indeed, in exposure assessments the monitoring of biomarkers is preferable over the evaluation of food contamination, due to variations in food preparation methods, food intake, contamination level, intestinal absorption, toxin distribution, and excretion leading to individual variations in toxin exposure that are more readily measured with a biomarker (Shephard et al., 2007). This primacy is also maintained over the air contamination. Owing to the limitations in sampling, collecting, and analytical, authors have gradually combined the air measurements with the level in body fluids, namely blood, to evaluate the exposure (Iavicoli et al., 2002).

The approach involving biomarkers does not represent a new pathway replacing the classic evaluation of dietary intake and risk assessment, but it has to be considered as a means of confirming dietary intake data obtained in the traditional way and, furthermore, of supplying the most relevant information on the real impairment to human health attributable to the toxic substances (Miraglia et al.,

1996). In Table 1 a synopsis comparison is made regarding OTA internal and external doses in exposure assessment. Although some heterogeneity of the OTA levels found in biological fluids may be explained by differences in the detection limit of the employed methods, they can also result from variations in dietary habits, which fluctuate between individuals and render additional importance to the use of biomarkers (Skaug et al., 2001). If properly validated, data on biomonitoring provide the ultimate evidence that exposure has taken place (Thuvander, 2001).

OTA biomarker profiling has been carried out focusing mainly on blood specimens. Nevertheless, more recently, attention has been drawn to the urinary biomarkers with the leading advantage of noninvasive procedure of collection. Although this is also true for milk specimens, the exposure can obviously only be assessed for lactating women, contrary to the urine specimens, which can be provided by the general population. Other promising biospecimens are also examined. Besides the parent compound, other biomarkers that have been

Table 1. Comparison of advantages and disadvantages of different monitoring methods in OTA exposure assessment.

Exposure assessment	Matrix	Advantages	Disadvantages
Internal dose	Blood	- Relatively high OTA levels: less sensitive methods required	- Invasive collection, involving medical personnel
			- Estimation of exposure: based on assumptions (bioavailability and plasma clearance)
			- Short-term biomarker
			- High within-subject and seasonal variability: limited use at the individual level
	Urine	- Ease, fast, and noninvasive collection	- Lower levels than serum: sensitive and accurate methods mandatory
			- Incomplete knowledge about indication of time
	General	- Total OTA exposure: reflects exposure by all routes, from all sources and bioavailability of the mycotoxin	- Lack of specific and validated markers, compliance difficulties, and/or missing information on the toxicokinetics of OTA in humans
		- Only one type of sample needs to be analyzed (through estimated daily intake, several samples would have to be considered)	
		- Enhanced individual and group risk assessments	
External dose	Food	- Determining the potential risk associated with exposure of a particular foodstuff or type of diet	- Assessment of exposure through analysis of foods particularly problematic: wide range of food types, the sporadic occurrence, and the low levels at which OTA is found
			- Problem with obtaining a representative sampling
			- Several types of foods need to be analyzed to retrieve a proportional contribution to the total estimated exposure (in relation to one for biomonitoring)
			- Only population-based estimated exposure given the heterogeneous contamination of food it is not always indicative of potential individual exposures
			- Changing nature of food consumption patterns within individuals over time, that is from day to day, season to season and also between different life periods (age, marital/family status, illness, income)
			- Up-to-date, correct, and available food consumption of the general population or risk group data mandatory
			- Measurement of exposure through one or a group of foodstuffs cannot always provide a full picture of human exposure
	Air	- Possible significant source of exposure, in domiciliary and occupationally settings	- Neglects the main source of exposure: ingestion
	General	- Necessary to quantify the increment of exposure derived from particular sources or media and identify the strategies necessary for exposure reduction and source control	- Considers only one source of exposure, and neglects multiple sources of exposures
			- Measures the external dose, which according to the individual susceptibility or type of exposure may vary

monitored in biological matrices are also looked at. The characteristics, advantages, limitations, and current knowledge on OTA exposure and effect biomarkers are focused in this review, in an attempt to gather the most relevant and recent information on the topic. An effort is also made to relate individual, regional, and seasonal features with OTA biomarkers from the information available.

2. Biomarkers of exposure

Biomarkers of exposure are measured, in body fluids or tissues, as the molecule itself, a metabolite(s), or a product of a reaction with a biological molecule. Biomarkers of exposure can be divided into (a) markers of internal dose and (b) markers of effective dose. The former is an indication of the occurrence and extent of exposure of the organism, whereas the latter is an indication of the extent of exposure of what is believed to be the target molecule, structure, or cell (Timbrell, 1998).

2.1.Biomarkers of internal dose

These biological markers indicate that exposure to a particular compound has taken place by measuring the compound or its metabolite(s) in body fluids (Timbrell, 1998). In this review, particular attention is paid to blood, urine, and milk specimens, as these are the most studied ones. OTA in serum, urine, and milk qualifies as a biomarker of exposure due to its slow excretion from the body, with a half-life in blood of several weeks. A major drawback is still the incomplete knowledge of OTA pharmacokinetic profile (Larsen, 1995).

2.1.1.Human OTA blood biomarkers

The most studied approach for biomonitoring of OTA exposure evaluation is certainly the measurement of the mycotoxins concentrations in blood specimens (Castegnaro et al., 2006). OTA is found in human blood due to the long elimination half-life, as consequence of its binding to plasma proteins, its enterohepatic circulation, and its renal reabsorption (Roth et al., 1988; Dahlmann et al., 1998). It is considered to represent a convenient biomarker of the exposure during previous weeks—a work with one human volunteer calculated the half-life of OTA in plasma to be 35 days in humans. Above and beyond being a short-term biomarker, OTA blood levels show high within-subject variability, as shown by the repeated samples collected 1 year apart, by Palli et al (1999), who observed almost no correlation between them. This finding suggests thus a restricted use of this biomarker at the individual level, despite its usefulness to characterize and assess the relative exposure of a population or subgroups of subjects, particularly in prospective studies storing blood samples taken at baseline, well before possibly related outcomes occur. This biomarker presents another weakness: it is considered of less value, since there is a lack of reliable information on the toxicokinetics of OTA in humans (MAFF, 1999; Palli et al., 1999; JECFA, 2008). Furthermore, blood samples are collected by an invasive procedure that involves medical personnel and inherent difficulties to the sampling from the study population. Thus, some studies make use of regular blood donation campaigns to assemble the samples from those areas. The results of this type of studies should be, however, carefully extrapolated to the general population, from which the blood donors emerge, since this type of study population is not representative of the whole population, as children and elder people do not donate blood, for instance (Coronel et al., 2009).

Through the use of this biomarker a possible active placental transport was pointed out by the double levels in fetal serum in relation to the maternal level as reported by Zimmerli and Dick (1995). It is interesting to note that a gradient between foetuses and their mothers also exists for phenylalanine for which mean ratios in the range of 1.2 to 1.9 have been determined (Zimmerli and Dick, 1995). A later study of Postupolski et al. (2006) determined similar differences between maternal and umbilical cord serum, 1.14 and 1.96 ng/ml, respectively (mean ratio of 1.96). When compared to the human blood concentrations, OTA concentration in human milk is lower (≤0.1), as determined in the study of Breitholtz-Emanuelsson et al. (1993a). More recently, Postupolski et al. (2006) found an OTA concentration ratio between maternal blood serum and the milk of 0.0058 on average. The ratios of OTA concentration in serum to whole blood are 2.0 ± 0.1 in humans, 1.9 ± 0.1 in pigs, and 1.7 in rats, as reported by Zimmerli and Dick (1995). In agreement is the mean ratio (2.0 ± 0.7) obtained in humans by Lino et al. (2008). These higher serum OTA levels, explained by the specific and strong bindings between OTA and serum albumin, in addition to cleaner extracts, are the main criteria for the use of serum or plasma as more suitable matrices in comparison to whole blood (Zimmerli and Dick, 1995; Curtui and Gareis, 2001; Lino et al., 2008).

OTA in blood specimens has been used extensively in epidemiological studies, but more systematically investigated since 1982, when the possibility that OTA could act as a main factor in the etiology of BEN, a particular form of nephropathy occurring in the Balkan peninsula (Hult et al., 1982; Petkova-Bocharova et al., 1988), prompted the International Agency for Research on Cancer (IARC) to investigate the presence of OTA in sera of the Balkanise population from both endemic and nonendemic areas. In the following years, researchers in several other countries developed similar studies aimed at the evaluation of OTA in biological fluids (Miraglia et al., 1996), showing continuous and widespread human exposure to this mycotoxin (Table 2). Roughly, OTA levels were found to be in the ng/ml range, often with frequencies among population extending from 20% to 85%, sometimes reaching 100% (Palli et al., 1999). Furthermore, the European Food Safety Authority (EFSA, 2006) highlights a recently observed tendency of OTA blood serum level decrease in the human population, attributing it to an increased awareness of the potential adverse health effects of

mycotoxins, and to the preventive measures (establishing of maximum permissible levels in food commodities) taken by various countries, including the community regulations to be applied in all European Union (EU) member states.

Comparison of the incidence and mean levels between different studies is in fact difficult, as the limits of detection or limits of quantification of the analytical methods are different in each case (Lino et al., 2008; Coronel et al., 2009). Moreover, little is known about the influence exerted by individual and social characteristics or even by season. Several studies addressed the topic, namely concerning anthropometrical parameters, although they largely failed to ascertain a statistically significant and defined association. That is the case of the parameter gender, for which most of the studies, even based on unequal sex ratios (Sangare-Tigori et al., 2006), considering healthy individuals (Breitholtz et al., 1991, Thuvander et al., 2001; Coronel et al., 2009; Erkekoğlu et al., 2010) or with underlying nephropathic conditions (Grosso et al., 2003; Sangare-Tigory et al., 2006; Dinis et al., 2007), no correlation was observed. However, in an epidemiological study conducted in central Italy, for estimation of the intrasubject variability (two samples collected with a 1-year interval), Palli et al. (1999) detected a strong positive correlation between OTA concentrations and adult male gender. It had already been previously established that male adults of the Southern Switzerland presented higher serum levels when compared to women (Zimmerli and Dick, 1995). The same was detected by Jonsyn-Ellis (2007) when studying children, for which significant difference was observed between boys and girls, although no reason for this difference was pointed out. More recently, amongst healthy Tunisian participants, Karima et al. (2010) detected a significantly higher mean value of 0.77 ± 0.10 ng/ml for men than for women, with 0.24 ± 0.03 ng/ml, although with comparable proportion of positive samples (27.7% and 28.3%, respectively). Different metabolism of OTA for each gender may explain this difference (Coronel et al., 2009). Indeed in rats, the higher susceptibility of males to OTA genotoxicity and carcinogenicity was shown to be related to the expression of toxifying enzymes (cytochrome P450 2C11, cyclooxygenase 2 [COX2], cytochrome P450 3A4) in contrast to females, along with a large number of metabolites formation in male rat kidney compared to female (Pfohl-Leszkowicz et al., 1998; Manderville and Pfohl-Leszkowicz, 2008). However, this pattern is not always constant. For instance, in a Chilean city (Muñoz et al., 2006), women (0.88 ± 0.40 ng/ml) presented significantly higher levels than men (0.51 ± 0.38 ng/ml). But in a second studied city, comparable level between genders existed, leaving unexplained the differences registered in the first city, as a one time situation or with an anthropometric, social, and/or dietary reason.

Like the factor gender, age does not appear to be a robust determinant of OTA exposure. In the sampled and analyzed population of Lebanon (Assaf et al., 2004), Japan (Ueno et al., 1998), Italy (Palli et al., 1999), Tunisia (Karima et al., 2010), and Spain (Jimenez et al., 1998), no significant variation was detected in either the incidence or the levels of toxin found among the different ages; even with underlying nephropathic conditions (Jimenez et al., 1998; Grosso et al., 2003). Yet, in other studies, although no significant differences are detected, a trend towards a higher frequency of detection or contamination levels is perceived with increasing age. Indeed, when studying children, Jonsyn-Ellis (2007) reported a higher tendency towards a higher frequency of detection in older children, although it failed to reach significance. Perhaps due to the long half-life time of OTA in human blood, one continuously exposed could have higher blood concentrations of OTA with time (Sangare-Tigori et al., 2006). Similarly, Coronel et al. (2009) found that the higher the age of the participants, the higher the OTA levels in plasma. This tendency to find higher OTA levels as the age increased (explicitly over 45 years) matched the trend also previously observed by Gilbert et al. (2001) in the UK (between 30 and 44 years), Filali et al. (2002) in Morocco (between 40 and 50 years), Pacin et al. (2008) in Argentina (between 51 and 60 years), and Lino et al. (2008) in Portugal (between 40 and 60 years). By analysis of different trends between urine and plasma levels with age, Gilbert et al. (2001) suggested that the efficiency of OTA removal from the body decreases with age, leading to higher plasma levels. In Turkey, although no significant differences were observed between the age groups, regardless of season or region, the highest OTA concentration was determined in summer for both regions, in the children living in Black Sea Region (0.877 ± 0.153 ng/ml) and in the elderly population living in the Mediterranean region (0.405 ± 0.122 ng/ml) (Erkekoğlu et al., 2010). Still attention should be drawn to three aspects: first, there are limited studies intending to correlate OTA blood levels and age; second, the majority of the existing ones involve only or mainly adult participants; and third, the age grouping differs among them, thus impairing comparisons and preventing establishments of risk groups.

As for other anthropometric indices, such as body mass index, weight, height, etc., because they are less studied, even less knowledge of their impact on OTA levels or incidence exists. Nonetheless, scattered studies on blood OTA levels evidenced a positive association with height and a negative association with blood pressure (either systolic or diastolic) (Palli et al., 1999). The absence of persistent significant differences between most of the anthropometric parameters strongly suggests that the source of OTA is transversal to the population, which means that, considering that the major source of exposure is the ingestion of contaminated food, a common dietary foodstuff is probably implicated (Ueno et al., 1998).

The geographical variations observed in blood OTA levels are also probably resultant from differences in the ingestion of contaminated foodstuffs, namely in respect to dietary habits or origin of the food consumed. For

Table 2. The occurrence and level of OTA (ng/ml) in recent studies in human blood specimens.

Location [time]of collection	Blood specimen	Source	Incidence (%)	LOD (LOQ)	Range of concentration	Mean	Reference
Sweden [1989]	Plasma	Plasma donors	38/297 (12.8%)	0.3 (0.8)	n.d.*–6.7	—	Breitholtz et al., 1991
Japan, Tokyo [1992; 1996]	Plasma	Student, healthy	156/184 (85%)	0.004 (0.02)	n.d.–0.278	0.068	Ueno et al., 1998
Egypt	Serum	- ESRD medical treat.†	- 4/11 (36.4%)	—	- n.d.–3.75	- 1.01 ± 1.56	Wafa et al., 1998
		- ESRD dialysis‡	- 4/15 (26.7%)		- n.d.–2.17	- 0.34 ± 0.69	
		- Renal transplant recipients	- 2/15 (13.3%)		- n.d.–6.30	- 0.47 ± 1.62	
		- Patient nephrotic syndrome	- 8/15 (53.3%)		-n.d.–10.15	- 2.19 ± 3.07	
		- Urothelial tumors	- 3/15 (20%)		- n.d.–5.57	- 0.52 ± 1.46	
		- Potential kidney donors	- 2/15 (13.3%)		- n.d.–0.91	- 0.08 ± 0.24	
		- Healthy group	- 0/25 (0%)		- 0	- 0	
Spain [1996; 1998]	Plasma	- Healthy donors	- 40/75(53.3%)	0.52	n.d.–4.0	- 0.71- 1.97	Jiménez et al., 1998
		- Hemodialysis patients	- 56/72 (77.8%)				
Croatia, Zagreb[1997; 1998]	Plasma	Healthy	88/110 (80%)	0.2	n.d.–1.3	0.19	Domijan et al., 1999
Italy, Tuscany[1994; 1996]	Serum	Adult, healthy	134/138 (97.1%)	—	n.d.–2.84	0.56	Palli et al., 1999
Turkey, Sparta	Serum	- Healthy	- _/40	0.2	-n.d.–1.43	-0.44	Özçelik et al. 2001
		- Patients with urinary disorders	- _/93		-n.d.–5.5	-0.74-2.1	
Croatia [1997; 1998]	Plasma	Blood donors	468/983 (48%)	0.2	—	0.30	Peraica et al., 2001
-Norway	Plasma	Blood Donors	393/393 (100%)	0.01	—	- 0.18	Thuvander et al., 2001
-Sweden [1997; 1998]						- 0.21	
Morocco [2000]	Plasma	Healthy	185/309(60%)	0.1	n.d.–6.59	0.29	Filali et al., 2002
Tunisia, Sahel	Serum	Hospitalized patients:		0.1	—		Grosso et al., 2003
		-non-UTD¶	-62/62 (100%)			-0.53 ± 1.00	
		-UTD	-38/47 (80.9%)			-0.99 ± 1.28§	
Bulgaria, BEN area	Serum	Healthy inhabitants:		0.1			Petkova-Bocharova et al., 2003
		- Gorno Peshtene	- 5/5 (100%)		- 0.2–1.6	-0.67 ± 0.24	
		- Beli Izvor	- 11/11 (100%)		- 0.1–10.9	-2.01 ± 0.54	
Lebanon [2001; 2002]	Plasma	Healthy	82/250 (33%)	0.1	n.d.–0.87	0.17	Assaf et al., 2004
Tunisia, Monastir	Plasma	- Healthy	-_/20 (71%)	—	- n.d.–7.5	- 2.6 ± 2.3	Hassen et al., 2004
		-CIN patients‖	-_/20 (93%)		- n.d.–140.5	- 44.4 ± 19	
		-Nephro patients#	-_/40 (83%)		- n.d.–73.19	- 8.11 ± 6	
		-Healthy	-_/20 (62%)		- n.d.–3.2	- 1.22 ± 1.2	
		-CIN UA**	-_/20 (100%)		- 18.4–171.25	- 50.4 ± 8.2	
		-CIN KA††	-_/20 (78%)		- n.d.–29	- 12.36 ± 4.4	
Pakistan, Karachi	Plasma	-Bladder cancer patients	-87/96 (90.6%)	0.03	- n.d.–3.41	- 0.33	Aslam et al., 2005
		-Healthy control group	-30/31 (96.8%)		- n.d.–1.24	- 0.31	
Czech Republic[1994; 2002]	Serum	Adult, healthy	2077/2206 (94.2%)	0.1 (0.05)	n.d.–13.7	0.28	Ostry et al., 2005

Table 2. continued on next page

Table 2. Continued.

Location [time]of collection	Blood specimen	Source	Incidence (%)	LOD (LOQ)	Range of concentration	Mean	Reference
Chile	Plasma	Blood donors	62/88 (70%):	0.1 (0.4)			Muñoz et al., 2006
- Colbún			- 54%		- n.d.–2.75	- 0.44	
- Tágua[2004]			- 91%		- nd.–2.12	- 0.77	
Côte-d'Ivoire, Abidjan [2001; 2002]	Serum	-Healthy regular blood donor	22/63 (34.9%)	—	n.d.–5.81	0.83	Sangare-Tigori et al., 2006
		-Nephropathy patients undergoing dialysis	8/39 (20.5%)		n.d.–2.42	1.05	
Sierra LeoneNjala	Serum	5- to 14-year-old children from a single school	131/25 (19%)	—	n.d.–60	2.4	Jonsyn-Ellis, 2007
Portugal	Serum	Hemodialyzed patients		(0.05)			Dinis et al., 2007
- Coimbra			-50/50 (100%)		-0.12–1.52	- 0.50 ± 0.29	
- Aveiro[2002; 2003]			-45/45 (100%)		-0.15–1.03	- 0.49 ± 0.22	
Portugal, Center[2002]	Serum	Healthy population:		(0.1)			Lino et al., 2008
		- Coimbra	- 29/29 (100%)		-n.d.–0.96	-0.42 ± 0.18	
		- Verride	- 31/31 (100%)		-n.d.–2.49	-0.78 ± 0.53	
		- Ereira	- 44/44 (100%)		-n.d.–1.91	-0.44 ± 0.31	
Argentina	Plasma	Blood donors		0.012 (0.019)	—		Pacin et al., 2008
- Mar del Plata [2004]			-127/199 (63.8%)			-0.15	
- G. Rodriguez [2005]			-147/236 (62.3%)			-0.43	
Tunisia, Tunis [2005]	Serum	Non-nephropatic individuals	11/40 (27.5%)	0.1 (0.2)	n.d.–11.98	0.73 ± 2.35	Ghali et al., 2008
Spain, Lleida [2008]	Plasma	Blood donors	275/279 (98.6%)	0.075 (0.23)	n.d.–8.68	0.86 ± 1.07	Coronel et al., 2009
Turkey (Ordu; Antalya) [2007; 2008]	Serum	Healthy population:		0.025			Erkekoğlu et al., 2010
		- Winter	- 92/120 (76.7%)		-n.d.–0.887	0.137 ± 0.013	
		- Summer	- 116/119 (97.5%)		- n.d.–1.496	0.312 ± 0.034	
Tunisia	Serum	Healthy population	29/105 (28%)	0.1 (0.2)	n.d.–3.43	0.49 ± 0.67	Karima et al., 2010

*n.d. = nondetected; †ESRD medical treat. = ESRD (end-stage renal disease) patients under conservative medical treatment; ‡ESRD = (end-stage renal disease) patients under dialysis; ¶UTD = urinary tract disease; §excluding cancer patients (0.26 μg/L); ‖CIN of unknown etiology; #chronic nephropathies of known etiologies; **CIN of unknown etiology; ††CIN of known etiology.

example, as reported by Breitholtz et al. (1991), among three Swedish districts, the insular one (Island of Visby), thus more dependent on local products, registered the highest incidence and level of contamination. Previous surveys also demonstrated the highest levels of OTA in swine blood from this island. Although it does not explain directly the difference in human exposure (beyond the carryover risk that swine food products may pose), it points out to a common human and animal dietary source, most probably cereals and their derivatives locally grown or stored. Regional differences were also reported in Lebanon (Assaf et al., 2004), with higher blood OTA levels in the periphery (South of the country and Bekaa valley, 50% and 47%, respectively) compared to the capital region (19%) attributed to specific local alimentary habits. In the Buenos Aires province, the regional differences observed were attributed to a lower socioeconomical level in the most affected region, which could imply the intake of lower-quality foods, thus more contaminated (Pacin et al., 2008). In Spain no differences between the localities were found, as expected by the immediacy shared among them (less than ~100 km), which does not result in sufficient differences in dietary habits (Coronel et al., 2009). In Tunisia, Karima et al. (2010) found significantly higher levels in the Sahal region (0.80 ± 0.14 ng/ml), as compared to the remaining central (0.67 ± 0.13 ng/ml), northern (0.23 ± 0.04 ng/ml), and southern (0.19 ± 0.04 ng/ml) regions studied. Previously, in the same country, different OTA levels had already been found, altering with location, dietary habits, and system of food storage and/or typical weather (Maaroufi et al., 1995, Grosso et al., 2003). Usually rural populations show higher mean OTA plasma levels, as reported in France (Creppy et al., 1991) and Portugal (Lino et al., 2008). The authors' rationalization included not only variations of climate and humidity levels, but also differences in consumption habits, namely alcoholic beverages, and ingestion of locally produced food, which significantly influences the OTA exposure.

Furthermore, seasonal variations were also reported. In Italy, Palli et al. (1999) found a strong association with the season in which blood samples were obtained, with summer values higher than autumn values. Indeed, in said study, height and season of blood collection were the strongest determinants of OTA levels and, taken together, accounted for about 16% of the variance of OTA. Two main reasons were pointed out for the higher values found in blood samples collected during the summer. The first related to particular climatic conditions and the other related to seasonal variation in the country dietary and drinking habits, which then account differently to exposure. In agreement, Erkekoğlu et al. (2010) reported statistically significant differences between summer and winter samples in the Turkish regions of the Mediterranean and the Black Sea. This increase of summer OTA levels was calculated as 3 times in children, 2 times in adults, and 2.5 times in elderly. Although no significant regional differences on the mean concentration of OTA in the same season was found, the highest values were detected in the Black Sea region, fact attributed to the inhabitants dietary habits—characterized by a higher consumption of cereals, namely corn and its derivates, along with the favorable local typical weather. In inhabitants from five studied cities of Croatia, the lowest frequency of positive samples and the lowest mean concentration of OTA were found in December (0.19 ng/ml), whereas the highest mean concentration was found in June (0.39 ng/ml) (Peraica et al., 2001). The same pattern of significant seasonal variation was previously observed the Croatian city of Zagreb, throughout the four collection periods (March, June, September, and December); the mean concentrations (0.27, 0.26, 0.16, and 0.08 ng/ml) and frequency of positive samples (64.6%, 58.0%, 44.0%, and 12.0%) reached a peak in March, after which they kept falling (Domijan et al., 1999). In Northern Spain, significantly lower levels were found during the months of June and October in both healthy and patients undergoing hemodialysis (Jimenez et al., 1998).

The presence of OTA in food samples from areas of Bulgaria where BEN and a high incidence of urinary tract tumors (UTTs) occurred was reported in the mid-1980s (Petkova-Bocharova and Castegnaro, 1985). Soon after, some authors found a higher mean value of OTA in a group of patients with BEN and the associated UTT, compared to groups of healthy people (Petkova-Bocharova et al., 1988). They concluded that the prevalence of the presence of OTA in the blood of people with BEN/UTT supported the hypothesis that this nephrotoxic and carcinogenic mycotoxin is involved in the etio-pathogenesis of these diseases (Özçelik et al., 2001). However, it is not clear how relevant current OTA levels are in view of the half-life of OTA in human serum (approximately 35.5 days). In addition, according to Palli et al. (1999), high OTA levels in patients with chronic renal failure might be due to a reduced clearance, which, according to the same authors, could be clarified with prospective studies using baseline OTA levels.

Impaired glomerular function, evidenced by proteinuria, in addition to enzymuria and hyalinization of glomeruli, is observable in patients suffering from BEN (Pfohl-Leszkowicz et al., 2002). However, recent studies suggest that the damages of glomeruli after OTA exposure described in BEN context are probably not based on functional changes of mesangial cells (Schwerdt et al., 2009). Within the kidney, the tubules seem to be the structure most affected by OTA. Indeed, in proximal tubule cell, Sauvant et al. (2005) demonstrated OTA-induced phenomena typical for chronic interstitial nephropathy, such as loss of cells and epithelial tightness, necrosis and apoptosis, as well as markers of inflammation, fibrosis, and epithelial-to-mesenchymal transition. Moreover, OTA was shown to induce all of the three mitogen-activated protein kinase (MAPK) pathways. Differential activation of MAPK might also serve as mechanistic explanation for the renal toxic action described for OTA (Sauvant et al., 2005). Although some

early results have indicated that contamination of food by OTA may be more widespread in rural endemic areas than in nonendemic areas, this is not entirely reflected by markedly elevated blood concentrations of OTA (Table 2). Indeed, a number of studies (Radić et al., 1997, Peraica et al., 1999, Petkova-Bocharova et al., 2003) verified high levels of OTA blood contamination in BEN areas; nonetheless, blood concentrations of OTA in the same range as those observed in endemic areas, i.e., northwestern Bulgaria, southeastern Romania, and ex-Yugoslavia (Serbia, Croatia, Bosnia and Herzegovina, Slovenia, and the former Yugoslav Republic of Macedonia), have been found in countries with no history of endemic nephropathy. Furthermore, human blood concentrations of OTA, even in areas with relatively high dietary exposure, are at least 2 orders of magnitude below the mean concentration of OTA in the blood of rats that is known to cause nephrotoxicity and kidney tumors with long-term treatment (Mally et al., 2007). Rizzo et al. (2002) explain this small difference with the concurrent contamination of OTA-contaminated food with additional mycotoxins, such as penicillic acid. It is also important to note that significant changes in lifestyle, migration pattern, food production, processing, and supply have taken place in the BEN endemic area, which might justify the observed trend towards a decrease in BEN incidence (Abouzied et al., 2002).

For the resemblance between BEN and a more recently described disease, CIN of unknown etiology in North African countries, namely Tunisia and Egypt, several investigations were locally conducted to analyze the role of OTA. With that objective, Hassen et al. (2004) demonstrated an association between high blood OTA levels, high β_2-microglobulinuria, and the CIN of unknown etiology. Compared to the remaining groups, healthy controls and other chronic nephropathic patients of known etiologies, patients with CIN of unknown etiology presented higher incidence (71%, 83%, and 93%, respectively), average level (2.6, 8.1, and 44.4 ng/ml, respectively) and maximum values (7.5, 73.2, and 140.5 ng/ml, respectively). The significantly higher levels in the last one discard the possibility that OTA is accumulated in patients regardless of the type of nephropathy, even though with the same glomerular filtration level (as the two last groups). The fact that the healthy donors show such an elevated incidence is likely due to the high prevalence of the mycotoxin in Tunisia, as indicated by the daily intake ranging from 4.7 to 130 ng/kg·body weight (bw)/day (Bacha et al., 1993), that takes into account cereals and cereal-derived foods as main contributors and blood OTA concentrations.

For the suspected role of OTA as the major etiological agent in BEN/UTT and CIN, several studies attempted to establish a relationship between blood biomarker and general nephropathic conditions, both in the endemic and nonendemic regions. The higher incidence (Algeria: Khalef et al., 1993) and average levels (Tunisia: Maaaroufi et al., 1995; Turkey: Özçelik et al., 2001) of OTA-positive samples reportedly found in the group of nephropathic individuals in several countries indicate that OTA may have a role in the human urinary pathology that affected the studied patients. Nevertheless, since urinary disorders develop during a long period of time, the OTA concentration in serum has to be monitored over many years if the role of OTA in human urinary pathology has to be evaluated (Özçelik et al., 2001). The reported OTA blood levels in nephropathic patients (Table 2) are variable, up to 85.3 ng/ml as detected in Tunisia (Maaroufi et al., 1995). Equally, the percentages of detection differ, up to 100% (Dinis et al., 2007; Grosso et al., 2003; Abid et al., 2003). These differences might be explained by three major clusters of rationales:

- The first cluster of rationales is related to the analytical quality, namely by different limits of detection of the employed analytical methodology (Grosso et al., 2003).
- The second cluster of rationales gathers the same factors that might influence the general population exposure rates, such as the climatic conditions, quality of the consumed foodstuffs, and type of food ingested. To verify if a correlation could be drawn between OTA-contaminated food ingestion and OTA blood and urine levels, Castegnaro et al. (2006) followed the same methodology employed by Gilbert et al. (2001) during 1 month in a Bulgarian high-incidence area of BEN and UTT. The results indicated that OTA blood level is relatively stable over a period of 1 month for a given individual and is regulated by urinary excretion. As long as the OTA food intake is relatively low, a variation of OTA food intake is not directly reflected by a variation of OTA blood concentration. The authors explained the results with the fact that the free fraction of toxin in plasma is less than 0.2% in all species including man as long as the binding sites are not saturated. Indeed, OTA is bound to proteins and stored in tissues, and thus could not be excreted directly by glomerular filtration. In organism, equilibrium exists between the bound and free forms of OTA in blood as well as in tissue. An increase of OTA blood concentration is rapidly compensated by increasing urinary OTA excretion, which brings the OTA concentration back to the original steady-state level in the blood (Castegnaro et al., 2006). Furthermore, from the outcome of the work of Gilbert et al. (2001), the significance of OTA levels in human plasma as a marker of OTA intake can be questioned: in the UK with low exposure to OTA, no correlation was found among healthy individuals.
- Lastly, and because these are patients presenting urinary disorders, the third cluster of rationale includes the type, degree, and evolution of the considered urinary disorder and, necessarily the type of treatment undergone by the patient, namely dialysis or transplant, which are part of the list of determining factors and thus contribute to variability. Jimenez et al.

a	b	c
$k_0 = Cl_p \times C_p/A$	$k_0 = 0.67 \times Cp/0.5$ (or $1.34 \times C_p$)	$k_0 = 0.99 \times Cp/0.5$ (or $1.97 \times C_p$)

Figure 2. Klaassen equation (a), and the two main versions (b and c) (modified from Gilbert et al., 2001; Scott, 2005).

(1998) reported significantly higher mean concentrations in patients undergoing hemodialysis (1.97 ng/ml) compared to healthy people (0.71 ng/ml), and suggested a mycotoxin accumulation in blood and tissues of patients with kidney disorders. Similarly, in Turkey, the mean serum concentrations of the toxin in all patients groups of urinary disorders were higher compared to a healthy control group (Özçelik et al., 2001). A significant difference was found between the mean concentrations of the groups of patients treated by dialysis (hemo- or peritoneal dialysis) and of the patients with renal stones or bladder cancer. A higher level of OTA in dialysis groups compared to the control, renal stone and bladder cancer groups could probably be explained by the reduced glomerular filtration rate of these patients, as also described previously by Breitholtz-Emanuelsson et al. (1994). If the results of Hagelberg et al. (1989) in monkeys are considered and furthermore extrapolated to humans, then elimination of OTA would be only through glomerular filtration. So, increased concentrations should be expected due to impaired kidney functions, leading to decreased filtration rates. Nevertheless, with dialysis, a reduction of the levels of blood contaminants such as OTA would be expected, since for example in hemodialysis, OTA would dialyze out of plasma into dialysis solution (Sangare-Tigori et al., 2006). So some authors (Dinis et al., 2007) found resembling levels between dialyzed and healthy individuals, indicating an effectiveness of the dialysis treatment in the elimination of contaminants of the organism. Higher mean levels would only be justified in limited conditions: (1) through a single exposure prior to sampling, via ingestion of contaminated foods; or (2) through a repeated exposure resulting from the fairly restricted diet in response to their nephropathy; and/or (3) the normal elimination via the kidney into urine of glucuronidated metabolites is blocked (Sangare-Tigori et al., 2006). So to fully discern the importance of the dialysis—effectiveness and duration—and the role of OTA in chronic interstitial nephropathy, an evaluation of the OTA levels would be of interest prior to the very first dialysis. Although the type of urinary tract disorder is essential, it does not always mean different levels in comparison to healthy members of the control group or population. That was the case of bladder cancer patients analyzed in Karachi (Aslam et al., 2005). In Egypt, significantly higher levels were found among end stage renal disease (ESRD) without dialysis than in the group of ESRD undergoing dialysis therapy, probably denoting clearance of OTA with dialysis, although OTA is known to be firmly protein bound. In the same study, the highest levels were detected in the group of patients presenting a nephrotic syndrome (2.19 ± 3.07 ng/ml), consistent with the previous clinical and experimental reports about OTA-induced tubulointerstitial lesions with atrophy of the proximal tubules, interstitial cortical fibrosis, and glomerulosclerosis due to heavy deposition of the toxin (Wafa et al., 1998).

All the cited studies based on OTA occurrence in blood matrices, considering both healthy and nephropathic individuals, provide good estimates of past and recent exposure to the parent compound, given its binding to serum proteins, which, however, does not reflect its biotransformation. A pilot study of Muñoz et al. (2010) attempted to develop a method that would simultaneously analyze OTA and a detoxification product, OTα, both in urine and plasma of 13 human volunteers. In both matrices, OTA and OTα had a frequency of detection of 100% (limit of quantitation [LOQ_{plasma}] 0.1 ng/ml); no evidence of OTA conjugates (including OTA glucuronide) was found, conversely to OTα conjugates (most likely OTα-glucuronide and possibly also OTα sulfate), which arose as major products. In plasma samples (without conjugate hydrolysis), OTA was the predominant form in women (0.23 ± 0.03 ng/ml) and men (0.26 ± 0.10 ng/ml), with higher although not statistically significant levels in the second. In contrast to OTA, the levels of its metabolite OTα increased considerably upon conjugate hydrolysis, in both urine and plasma samples. This is a clear indication of considerable levels of OTα conjugates occurrence in blood, with OTα aglycone accounting for about 10% of OTα total. The polar properties of OTα and its glucuronide conjugate result in a more efficient clearance from the blood circulation than OTA, as confirmed by these results.

Although nonconventional, other sources than foodstuffs, such as airborne sources, can present a risk of exposure to OTA (Duarte et al., 2009b). In this view, OTA levels in blood (plasma or serum) are not only suitable biomarkers of exposure in the general population but also in occupational settings (Muñoz et al., 2010). Occupational environments of central Italy, specifically green coffee, black pepper, nutmeg, and cocoa bean warehouses were studied (Iavicoli et al., 2002). Part of their male workers, occupied in processing and/or handling these commodities, were studied for OTA occurrence in plasma samples collected after the work shift. Statistically significant higher levels were found in exposed subjects (2.29 ± 0.99 ng/ml, range 0.94–3.28 ng/ml; $n = 6$) than in the control group (0.33 ± 0.25 ng/ml, range 0.03–0.95 ng/ml; $n = 23$), or other healthy European

populations (Table 2). Their levels are, however, similar to those of nephropathic patients treated by hemodialysis (2.1 ± 1.2 ng/ml) and peritoneal dialysis (1.9 ± 1.5 ng/ml) (Özçelik et al., 2001).

From OTA occurrence in blood it is possible to assess human exposure. The advantage of performing exposure estimation through OTA levels in blood is the fact that it is not necessary to be aware of the source of contamination involved—ingestion of contaminated food or inhalation of contaminated air. Additionally it requires a single determination per person, and saves all the problems associated with the food sampling methods and consumption data collection. It can, however, confer an underestimation of the intake, given the approximations in respect to toxicokinetic properties and the assumptions from which it stands (Miraglia et al., 1996; Coronel et al., 2009). Further studies on this underprovided knowledge of OTA kinetics are needed.

The Klaassen equation (Figure 2a) is currently in use to estimate the continuous exposure to OTA (k_0; ng/kg·bw/day) based on plasma concentration (C_p; ng/ml), plasma clearance (Cl_p; ml/kg·bw/day), and bioavailability (A). For the last two coefficients, assumptions are made: bioavailability, i.e., fraction of OTA taken up, is considered for most animals around 50% (Hagelberg et al., 1989); plasma clearance (Cl_p) considers only renal filtration rates, which might underestimate the plasma clearance, since no other route is considered, apart from renal filtration (Breitholtz et al., 1991). Two main values exist for the calculation of human renal filtration rate, and so two major versions exist for the same equation (Miraglia et al., 1996; Gilbert et al., 2001; Scott, 2005). The first (0.67 ml/kg·bw/day; Figure 2b) was calculated from the glomerular filtration rate of inulin (0.033 ml/min) and the free fraction of OTA, 0.02% (Hagelberg et al., 1989). The second one (0.99 ml/kg·bw/day; Figure 2c) resulted from a study with a single human volunteer that ingested ^{3}H-labeled OTA, which allowed the calculation of renal clearance of OTA as 0.048 ml/min, corresponding to 0.99 ml/kg·bw/day for a 70-kg person (Schlatter et al., 1996). When serum specimens are used, most authors use the determined concentrations as an approximation of plasma concentrations (Palli et al., 1999).

A number of tolerable daily intakes (TDIs) have been proposed by official organizations or simply by experts in the subject (Erkekoğlu et al., 2010). Kuiper-Goodman (1990) suggested a provisional TDI of 1.2–5.7 ng/kg·bw/day for a risk level of 10^{-5}. The Nordic Working Group on Food Toxicology and Risk Evaluation (1991) supported their calculations (5 ng OTA/kg·bw/day) with the carcinogenicity studies in adult rats with a safety factor of 5000. The same value for TDI was proposed by the Scientific Committee on Food (SCF, 1998) and the Upper Council for French Public Health (Conseil Supérieur d'Hygiène Publique de France, 1999).

More recently, EFSA (2006) proposed a TDI ~17.14 ng/kg·bw/day (120 ng/kg·bw/week) and JECFA (2007) reconfirmed the already previously established value of ~14.28 ng/kg·bw/day (100 ng/kg·bw/week) based on pig nephrotoxicity. More recently, Kuiper-Goodman et al. (2010) reevaluated the TDI as 4 ng/kg·bw/day. Examples of reported OTA plasma concentration–based estimated daily intakes (EDI; ng/kg·bw/day) in healthy populations are 1.69 ± 2.11 for Lleida donors, 1.75 ± 2.03 for men and 1.64 ± 2.19 for women (Coronel et al., 2009); 0.56 for Coimbra, 0.59 for Ereira, and 1.05 for Verride residents (Lino et al., 2008); 0.24 for Oslo and 0.28 for Visby residents (Thuvander et al., 2001); 1.46–2.15 UK citizens (Gilbert et al., 2001); 0.84 for Tágua and 1.40 for Colbún residents (Muñoz et al., 2006); 0.08 in Japan (Ueno et al., 1998); and 0.408 in summer and 0.182 in winter in Turkey (Erkekoğlu et al., 2010). A gap is evident between the reported EDI levels and the several recommended TDIs. Indeed, it is uncommon for a reported EDI to surpass these limits—such as one woman included in the study group of Coronel et al. (2009) or the subject from the study population of Palli et al. (1999). So, in general, these exposure estimates are not deemed to represent a health hazard. Furthermore, since some studies report mean EDI of OTA in BEN-endemic regions lower than that of the rest of Europe—0.53 ng/kg·bw/day (Peraica et al., 1999), 0.26 ng/kg·bw/day (Domijan et al., 1999)—the endemic nephropathy exists only in Balkan countries, probably because of a synergistic effect between OTA and penicillic acid and possibly other metabolites from ochratoxigenic fungal species (Rizzo et al., 2002). In fact, according to Stoev et al. (2001), the penicillic acid has an inhibitory effect on carboxypeptidase activity, an enzyme that is involved in the primary detoxification of OTA in the intestinal tract. In this way, penicillic acid could impair the natural detoxification of OTA, thus enhancing its toxicity. Furthermore, some studies provide evidences of enhancement of OTA carcinogenicity and genotoxicity by co-exposure to citrinin and fumonisins, as reviewed by Pfohl-Leszkowicz (2009). When comparing the EDI derived from the two available approaches, i.e., based on food consumption and food contamination data (EDI_{FOOD}) with the one based on plasma OTA concentrations (EDI_{PLASMA}), there is no clear tendency in the correlation of the estimations (Coronel et al., 2009). At first, Breitholtz et al. (1991) found a good agreement between the results obtained with the two methods of calculation (0.36 and 0.35 ng/kg·bw/day, respectively), as achieved by the duplicate diet study conducted by MAFF (1999) with equation in Figure 2b. This finding supports the accuracy of the values proposed by Breitholtz et al. (1991) on clearance and bioavailability of OTA in humans. The same is not true for the duplicate studies of Gilbert et al. (2001), according to which mean DI (Dietary intake) estimated from food consumed (0.94 ng/kg·bw/day) was lower than estimation from plasma concentrations (1.46 ng/kg·bw/day from equation in Figure 2b and 2.15 ng/kg·bw/day from equation in Figure 2c). Contrary results were reported by Thuvander et al. (2001) and Coronel et al. (2009), according to whom the estimated intake, calculated with food consumption and food contamination data retrieved from literature, resulted in values that were 5 and 2 times higher than the ones of DI based

on plasma OTA concentrations according to Equations 2b and 2c, respectively. Indeed, Thuvander et al. (2001) reported EDI_{FOOD} values of 1.3–1.4 and EDI_{PLASMA} values of 0.24–0.28 ng/kg·bw/day for the two studied populations; Coronel et al. (2009) reported EDI_{FOOD} values of 1.96 and EDI_{PLASMA} values of 1.69 ± 2.11 ng/kg·bw/day. The inconsistency is further demonstrated in the SCOOP report (Miraglia and Brera, 2002), according to which when comparing the results of the two ways of estimation, in Germany, Sweden, and Norway, the EDI_{PLASMA} values were lower than those deriving from EDI_{FOOD}, whereas the opposite result was obtained in Spain and in the UK. Possible reasons and sources of uncertainty that may account for these variable results with EDI_{FOOD} and EDI_{PLASMA} proposed ways of estimation, including (Thuvander et al., 2001; Miraglia et al., 1996; Coronel et al., 2009):

- Important dietary intake sources can be overlooked in the studies, or OTA exposure can occur via sources other than food alone, for example via airborne particles or spores from OTA producing fungi.
- Food consumption data derived from the literature may not be representative of the food that was actually consumed by the subjects prior to the study.
- Participants' food consumption data provided by the questionnaire, can lead to over- or underreporting of food consumption, and thus an over- or underestimation. This human bias occurs because it is based on an estimate of a recall period. The smaller this recall period is, the easier it will be for the participants to remember the consumed quantities more accurately; secondly, the influence of the changes of alimentary habits in the different seasons will thus expectedly be suppressed. Reported studies' recall period varies from 2 months (Thuvander et al., 2001) to a year (Coronel et al., 2009). However, considering the OTA half-life in plasma—35.5 days (Studer-Rohr et al., 2000), recall period for further studies should match with this.
- The calculation of OTA intake from plasma concentration, using the Klaassen equation, is a low estimate, as clearance (i.e., OTA elimination) is assumed to involve filtration only, and at an approximated rate, just as the bioavailability coefficient.
- Furthermore, slight variations may result from the fact that two versions can be used, and since the clearance rate is higher in one, a higher EDI_{PLASMA} value results. For instance, Thuvander et al. (2001) report plasma-based EDIs of 0.24–0.28 when using the Figure 2b equation, which are different from the 0.36–0.42 ng/kg·bw/day calculated with version 2c.
- Finally, normalization of the methods of data acquirement could be a good step for achieving comparable results (Coronel et al., 2009).

Recent comparative studies to correlate OTA blood levels and the dietary intake of the toxin raised misgivings about the reliability of this biomarker. Indeed, the analysis of blood levels for comparison with the analysis of food consumed by the volunteers (Gilbert et al., 2001), or with the analysis of food consumption data retrieved from questionnaires combined with use of analytical data from the literature (Thuvander et al., 2001; Muñoz et al., 2006; Coronel et al., 2009), failed to confirm an unequivocal association.

In the study of Thuvander et al. (2001) there was no correlation between plasma levels of OTA and the estimated total dietary intake of OTA based on consumption data and levels in food, neither was the plasma level of OTA correlated with the total amount of food consumed. The weak or absent correlation between food consumption patterns and plasma levels was explained as an expression of the widespread occurrence, along with heterogeneous distribution of the mycotoxin at rather low and occasional high levels in a large number of food items, resulting in a relatively small regular contribution to the exposure from each type of food, in different individuals or groups of individuals. This may also explain the lack of correlation found in analyses of the whole study population, whereas rather strong correlations were seen in a few cases for the subpopulations (i.e., for consumption of beer among women in Visby). It was furthermore shown that, at the present level of OTA exposure, there is no marked correlation between plasma levels and consumption of any specific type of food, although several types of food, including cereal products, wine, beer and pork, were all to some degree related to high plasma levels of OTA.

A similar study, but conducted in Chile, also did not produce any significant correlation between OTA levels in plasma and normal dietary consumption; a slight correlation or just a tendency with cereal, pork, and chicken consumption, changeable with the city and gender considered, was described. The specific food responsible for the OTA contribution was not identified (Muñoz et al., 2006). Likewise, in Spain, Coronel et al. (2009) also failed to identify the specific food or food groups responsible for OTA exposure, regardless of whether analysis was by gender, age, or location (Coronel et al., 2009).

Other possible explanation may be that food questionnaires may not include all the possible food groups, specifically the ones actually consumed by the study population, such as traditional consumed commodities, nor reflect traditional dietary habits, that may account locally as a significant source of exposure.

Therefore, additional studies on the kinetics and bioavailability of OTA in humans are needed to prove the applicability of the plasma level of OTA as a biomarker of dietary exposure (Thuvander et al., 2001).

2.1.2.Human OTA urine biomarkers

Although OTA levels in urine are very low in comparison to those in blood, they are regarded as a promising biomonitoring approach, more than ever with the advent of new methodologies that are more sensitive and accurate. It provides an additional advantage given the rapid

and easy noninvasive collection process (JECFA, 2008). Although currently no prospect exists that are designed for individual exposure calculation from OTA urinary levels, recent data (UK; Gilbert et al., 2001) sustain a stronger correlation between OTA urinary concentration and the level of consumption, in comparison with plasma concentration. This is justified by the fact that OTA in human blood samples is compromised by the long half-life of the toxin, for that a frequent dietary exposure will result in a steady-state concentration (EFSA, 2006). This stronger correlation with the level of consumption is perhaps one of the strongest advantages in using urine, given the major source of human exposure: contaminated food intake. Further studies, but conducted in a BEN area, tried to draw a correlation not only between OTA food intake and the contamination of urine and blood, but also with the amount of urine-excreted OTA collected in a whole day. By applying the same methodology as Gilbert et al. (2001), 16 human participants were studied and the results reported by Petkova-Bocharova et al. (2003) and Castegnaro et al. (2006). The authors describe an apparent correlation between average values of OTA intake, serum OTA, and urinary OTA excretion. They observed that an increase of OTA intake does not result in an immediate increase of OTA elimination, but only the week after high contamination. In contrast, when the OTA intake is low, OTA elimination is modulated by OTA blood concentration. Altogether, the results indicate that OTA blood level is relatively stable over a period of 1 month for a given individual and is regulated by urinary excretion. As long as the OTA food intake is relatively low, a variation of OTA food intake is not directly reflected by a variation of OTA blood concentration, as described previously (see Section 2.1.1).

According to a similar survey performed by Pfohl-Leszkowicz et al. (2007), OTA could be found in urine several days after OTA ingestion. In fact, OTA binding to serum proteins (>99%) facilitates its passive absorption in the nonionized form, but hinders its glomerular filtration (Gekle et al., 2005). OTA binds strongly to albumin (binding saturation above several hundred micrograms per milliliter of serum) (Schwerdt et al., 1999), but also strongly to other small proteins (20,000 Da), for which binding saturation is reached with an OTA concentration of 10–20 ng/ml (Stojkovic et al., 1984). The fraction of OTA bound to proteins can be released as soon as the free OTA fraction decreases. This delays elimination and thus increases the risk of accumulation of OTA in tissues (Pfohl-Leszkowicz et al., 2007).

The fact that high OTA intake is not always reflected by high serum OTA concentration and the highest serum concentration is not related to the highest OTA consumption renders blood of less use as a very recent exposure biomarker. In this aspect, although it lacks a correlation with serum levels, daytime OTA urine excretion is a more valuable tool than the urinary concentration (Petkova-Bocharova et al., 2003; Castegnaro et al., 2006).

The relationship between OTA excreted in urine with OTA intake is complex (Pfohl-Leszkowicz et al., 2007). In general, as demonstrated by Castegnaro et al. (2006), the elimination rate for humans is low (average value comprised between 20 and 80 ng/day) independently of the dose ingested, until the intake is below 100 ng/kg·bw/week. The excretion for low intake is in the same range as for rats. The OTA elimination increases dramatically and is multiplied by 10–50-fold for an average intake of 100 ng/kg·bw/week.

Despite the suggesting role of OTA, alone or with additive or synergistic effects from two or more mycotoxins (e.g., citrinin, penicillic acid), in the chronic renal diseases described in the Balkans (BEN) and North African countries (CIN), for the high contamination levels reported (Bacha et al., 1999; Nikolov et al., 2002; Sangare-Tigori et al., 2006: Pfohl-Leszkowicz et al., 2007), it is found in urine of study populations worldwide. In fact, and as summed up in the latest studies displayed in Table 3, the highest incidences of contamination, of 100%, were observed mutually in BEN-endemic (Petkova-Bocharova et al., 2003) and nonendemic regions, such as Italy (Breitholtz-Emanuelsson et al., 1994), UK (Gilbert et al., 2001), and Portugal (Duarte et al., 2010). As for the latter Portuguese study, of a nationwide nature, the total incidence of 92.2% observed was rather different for the one reported for a particular region, Coimbra (73.3%). In previous local studies carried out, frequencies varied from 70% (Pena et al., 2006) down to 43.3% (Manique et al., 2008). This frequency of contamination observed in Coimbra bears an evident resemblance to the one reported in the capital region (72.1%), Lisbon (Duarte et al., 2009a).

Regarding the average contamination levels, a range from 0.013 ng/ml in Hungary (Fazekas et al., 2005) up to 0.53 ng/ml in Southern Italy (Breitholtz-Emanuelson et al., 1994) was reported. With the exception of the value of 0.168 ng/ml reported by Petkova-Bocharova et al. (2003) in a BEN area, the remaining reported values fall in the lower range, in the same order of magnitude reported in Hungary. Lower values than reported for Hungary were recently observed by Domijan et al. (2009) in residents from a BEN-area village. Unfortunately, not all the studies supply with average values and limits of detection or quantification, which hinders additional comparisons. It is also important to underline the low levels at which the OTA occurs in urine, comparatively to blood, which may compromise results in the absence of a reasonably sensitive and accurate analytical technique. This happens because as Studer-Rohr et al. (2000) demonstrated on a human volunteer, only 42–54% of intact OTA (parent compound) was detected in urine, which might add importance to the alternative determination of OTA metabolites and/or conjugates in urine as exposure biomarkers. In the urine of rats, a total of 20 different metabolites of OTA, OP-OTA, OTB, OTA-OH, and OTα were detected (Li et al., 2000). In children, the metabolic product of OTA-4-hydroxyochratoxin A (4R-OTA) was detected with more frequency (reaching statistical significance) in girls than boys, although the difference was more pronounced during the rainy season than the dry season, by 52% versus

Table 3. Occurrence and levels (ng/ml) of OTA and/or its metabolites in urine in different countries.

Location [time] of collection	Biomarker	LOD (LOQ)	Sampled population	Incidence No. positive (%)	Range	Mean	Reference
Egypt	OTA		- ESRD medical treat.*	- 4/11 (36.4%)	- n.d.†-6.70	- 1.85 ± 2.82	Wafa et al., 1998
			- ESRD dialysis	- 1/11 (9.1%)	- n.d.–4.0	- 0.36 ± 1.21	
			- Renal transplant recipients	- 2/15 (13.3%)	- n.d.–1.36	- 0.12 ± 0.36	
			- Patient nephrotic syndrome	- 8/15 (53.3%)	- n.d-8.19	- 3.09 ± 3.47	
			- Urothelial tumors	- 1/15 (6.7%)	- n.d-4.64	- 0.36 ± 1.20	
			- Potential kidney donors	- 2/15 (13.3%)	- n.d.–3.42	- 0.26 ± 0.91	
			- Healthy group	- 0/25 (0%)	- n.d.–0.31	- 0.01 ± 0	
U.K.	OTA	0.01	Volunteers	46/50 (92%)	n.d.–0.058	—	Gilbert et al., 2001
Italy (South & North)	OTA	0.005	Healthy	22/38 (57.9%)	0.012–0.046	—	Pascale and Visconti, 2000
Italy (South)	OTA	0.05	Healthy	133/133 (100%)	n.d.–14	0.53	Breitholtz-Emanuelsson et al., 1994
Hungary	OTA		Healthy	54/88 (61%)	0.006–0.065	0.013	Fazekas et al., 2005
Bulgaria (Gorno Peshtene village)	OTA (0.004)		Healthy BEN area residents	5/5 (100%)	0.010–0.33	0.051	Petkova-Bocharova et al., 2003
Bulgaria(Beli Izvor village)			Healthy BEN area residents	11/11 (100%)	0.010–1.910	0.168	
Bulgaria [1984; 1990]	OTA		BEN/UTT patients	14/36 (38.9%)	0.005–0.604	—	Nikolov et al., 2002
			Healthy persons from BEN families	12/25 (48%)	n.d.–0.033	—	
			Healthy persons from non-BEN families in BEN villages	14/32 (43.8%)	n.d.–0.043	—	
			Healthy persons from non-BEN villages in BEN area	4/31 (12.9%)	n.d.–0.041	—	
			Healthy persons from non-BEN area	0/3 (0%)	n.a.‡	—	
Bulgaria	OTA	0.005	BEN/UTT patients and controls	61/152 (40%)	n.d.–0.03	—	Castegnaro et al., 1990
	4-hydroxy-OTA	0.015		0/152 (0%)	—	—	
Sierra Leone	OTA		Children (<5 years)	13/54 (24%)	n.d.–26.6	—	Jonsyn, 1999
	4-hydroxy-OTA			24/54 (44.4%)	n.d.–21	—	
	4-hydroxy-OTA		Boys (5–14 years)	78/231 (33.8%)	0.1–37	—	Jonsyn-Ellis, 2000
			Girls (5–14 years)	89/203 (43.8%)	0.1–33	—	
	OTA	0.2	Boys (5–14) years	55/231 (23.8%)	0.07–72	—	
			Girls (5–14) years	55/203 (27.1%)	0.08–148	—	

Table 3. continued on next page

Table 3. Continued.

Location [time] of collection	Biomarker	LOD (LOQ)	Sampled population	Incidence No. positive (%)	Range	Mean	Reference
Portugal, Coimbra [2004]	OTA (0.02)		Healthy	42/60 (70%)	n.d.–0.105	0.038	Pena et al., 2006
Portugal, Coimbra [2005; 2006]	OTA (0.007)		Healthy	13/30 (43.3%)	n.d.–0.208	0.019	Manique et al., 2008
Spain, Valencia [2005]			Healthy	25/31 (80.6%)	n.d.–0.124	0.032	
Croatia [2000]	OTA	0.005	Healthy non-BEN area	5/18 (28%)	n.d.–0.02	0.003 ± 0.005	Domijan et al., 2009
			Healthy BEN area	19/45 (43%)	n.d.–0.086	0.007 ± 0.014	
Croatia [2005]			Healthy non-BEN area	1/18 (6%)	0.01	0.005 ± 0.024	
			Healthy BEN area	8/45 (18%)	n.d.–0.015	0.001 ± 0.003	
Portugal, Lisbon [2007]	OTA (0.008)		Healthy women	15/21 (71.4%)	n.d.–0.055	0.027	Duarte et al., 2009a
			Healthy men	16/22 (72.7%)	n.d.–0.071	0.025	
			Total	31/ 43 (72.1%)	n.d.–0.071	0.026	
Portugal, Nationwide [2007]			Healthy women	81/88 (92.0%)	n.d.–0.069	0.021	Duarte et al., 2010
			Healthy men	62/67 (92.5%)	n.d.–0.068	0.023	
			Total	143/155 (92.2%)	n.d.–0.069	0.022	
Germany	OTA (0.05)	0.02	n.s.	13/13 (100%)	n.d.–0.14	0.07 ± 0.05	Muñoz et al., 2010
	$OT\alpha_{(aglycone)}$ (0.05)	0.02		13/13 (100%)	n.d.–4.70	1.14 ± 1.17	
	$OT\alpha_{(total)}$ (0.05)	0.02		13/13 (100%)	n.d.–7.12	2.88 ± 2.24	

*ESRD medical treat. = ESRD (end-stage renal disease) patients under conservative medical treatment; †n.d. = nondetected, ‡n.a. = nonapplicable.

Table 4. Selected worldwide studies on the occurrence of OTA (ng/ml) in human breast milk.

Location [time] of collection	LOD (LOQ)	Incidence (%)	Range (mean)	Sample (period)	Reference
Italy	0.2	9/50 (18%)	n.d.*–6.6	n.s.† milk	Micco et al., 1991
Sweden	0.01 (0.04)	23/40 (58%)	n.d.–0.04	n.s. milk	Breitholtz-Emanuelsson et al., 1993a
Sierra Leone	0.2	40/113 (35.4%)	n.d.–337 (7.9 ± 5.2)	n.s. milk	Jonsyn et al., 1995
Switzerland (north of the Alps)	(0.005)	4/40 (10%)	n.d.– 0.014	n.s. milk	Zimmerli and Dick, 1995
Italy	0.1	22/111 (19.8%)	n.d.–12	Milk from hospitalized and nonhospitalized mothers	Micco et al., 1995
Australia, Victoria	1.6	2/100 (2%)	n.d.–3.6	n.s. milk	Apostolou et al., 1998
Norway [1994]	0.01	38/115 (33%):	n.d.–0.13:	n.s. milk (3–4 days postpartum)	Skaug et al., 1998
- North coast		- 7/48 (14.6%)	- n.d.–0.056 (0.027)		
- Middle coast		- 11/19 (57.9%)	- n.d.–0.102 (0.049)		
- South east inland		- 20/48 (41.7%)	- n.d.–0.130 (0.043)		
Egypt, Cairo; Giza [1999; 2000]	—	3/10 (30%)	n.d.–15 (8.87)	n.s. milk	EI-Sayed et al., 2000
Norway, Oslo [1995; 1996]	0.01	17/80 (21%)	n.d.–0.182 (0.03)	Morning milk (4 weeks postpartum)	Skaug et al., 2001
Egypt [2000; 2002]	—	43/120 (35.8%)	n.d.–45.01 (21.06 ± 13.07)	n.s. milk	EI-Sayed et al., 2002
Italy, Lombardy [2000]	0.0005	198/231 (85.7%)	n.d.–0.057 (0.006 ± 0.008)	n.s. milk (3–4 days postpartum)	Turconi et al., 2004
Brazil, S. Paulo [2001; 2002]	(0.01)	2/50 (4%):	n.d.–0.02:	Human milk bank	Navas et al., 2005
- Winter		- 0/20 (0%)	- n.a.‡		
- Summer		- 2/28 (7.1%)	- n.d.–0.02 (0.015)		
Poland	—	5/13 (38%)	n.d.–0.17	n.s. (milk)	Postupolski et al., 2006
Egypt, Zagazig; Mansoura	—	36/50 (72%)	(1.89 ± 0.98)	Milk from healthy lactating women	Hassan et al., 2006
Martin, Slovakia	0.0048 (0.0144)	23/76 (30.26%)	n.d.–0.06	Milk from lactating women with hospitalized children (0–6 months postpartum)	Dostal et al., 2008
Italy	0.002 (0.005)	61/82 (74.4%)	n.d.–0.405 (0.030 ± 0.067)	Mature milk (30 days postpartum)	Galvano et al., 2008
Turkey, Ankara	0.01	75/75 (100%)	0.62–13	n.s. (milk)	Gürbay et al., 2009

*n.d. = nondetected; †n.s. = nonspecified; ‡n.a. = nonapplicable.

29% and 38% versus 37%, respectively. Similarly, ochratoxin B (OTB), which often co-occurs with OTA, had the differences of frequency between genders intensified during the rainy seasons, with 32% in boys and 44% in girls. However, it should be noted that the incidence of contamination was higher in dry season than in rainy season for both 4R-OTA (21% and 17.5%) and OTB (26.5% and 16.6%) (Jonsyn Ellis, 2000). The same 4R-OTA metabolite was not found in any of the 152 BEN/UTT patients and controls studied by Castegnaro et al. (1990).

Conversely, Castegnaro et al. (2006) detected, without quantify, 4-OH-OTA, along with OTα, in the urine of all Bulgarian participants enrolled in the study. More recently, in all the blood and urine samples of group of 13 volunteers, the presence of OTA and OTα was revealed. In urine samples (without conjugate hydrolysis), OTα was the major metabolite, with average aglycone concentrations (mean value 1.00 ± 0.37 ng/ml for women, and 1.31 ± 1.68 ng/ml for men), about 16–20 times higher than those for OTA. No OTA conjugates were found, i.e., the concentrations after enzyme (β-glucuronidase/sulfatase) treatment were not higher than those of OTA measured upon direct extraction. In contrast to OTA, the levels of its metabolite OTα increased considerably (about 5-fold) upon conjugate hydrolysis, in both urine and plasma samples. So in both matrices conjugates of OTα (glucuronide and/or sulfate) are major products, as confirmed by mass spectrometry detection (Muñoz et al., 2010). The absence of OTA glucuronide in urine, which seems to suggest the absence of an glucuronidation pathway, was in line with the results of Valenta (1998) and Vatinno et al. (2007), but in contradiction with those of Pena et al. (2006) that, based on indirect evidence, suggested the presence of OTA glucuronide in human urine. Muñoz et al. (2010) reported a considerable interindividual variation in the urine levels of total OTα, which was assumed to be related to interindividual differences in OTA degradation by gastrointestinal microbial or mammalian hydrolytic enzymes, known to convert OTA to OTα. Although it is true that because OTA occurs at quite low levels in urine, the analysis of OTα may increase sensitivity and facilitate biomonitoring, the reported substantial interindividual variation on human ability for OTA detoxification might greatly compromise the use of OTα as an alternative or simply additional biomarker.

On the matter of social, seasonal, or anthropometric influences, fewer to no answers are available. In Sierra Leone no correlation was found between nutritional status and the prevalence of OTA in urine samples of children (Jonsyn-Ellis, 2000). In the same study, a higher incidence of OTA contamination during the collection period of dry season comparatively to the rainy season (correspondingly 14.5% and 10.8%) and in girls comparatively to boys (correspondingly 27.1% and 23.8%) was observed. Conversely, no major differences were observed for OTA levels in urine from adult men (0.09 ± 0.05 ng/ml) and women (0.05 ± 0.05 ng/ml) by Muñoz et al. (2010). Similarly, in Portuguese adults no statistically significant differences were detected between male- and female-provided samples, either on incidence (92.5% versus 92.0%) or mean level (0.023 versus 0.021 ng/ml) (Duarte et al., 2010). This was in agreement with previous results obtained for the frequency (72.1% versus 71.4%) and mean value of contamination (0.025 versus 0.027 ng/ml) of the Lisbonian male and female inhabitants for the same period (Duarte et al., 2009a). Previously in Portugal, total samples provided by male participants also presented higher frequency and mean level (79%; 0.039 ng/ml) than female participants (58%; 0.037 ng/ml), although it didn't reached significance, with the exception of the age group 20–39 years (Pena et al., 2006). In the same study area, center of Portugal, morning samples provided by male participants presented higher incidence of contamination and mean level (53.8%; 0.026 ng/ml) than female ones (35.3%; 0.013 ng/ml). Simultaneous determination on a study population from Valencia did not follow the same pattern, since little to no differences were found between the male and female genders (80.0% versus 80.9%; 0.029 versus 0.033 ng/ml) (Manique et al., 2008). According to clinical observations in humans and studies in experimental animals in vivo and in in vitro models, renal structure and functions under various physiological, pharmacological, and toxicological conditions are different in males and females, and these differences may be related to the sex-hormone-regulated expression and action of transporters in the apical and basolateral membranes of nephron epithelial cells. The renoprotective effects of estrogens were further demonstrated in various pathophysiological conditions and diseases in humans and experimental animals, including nephrotoxicity induced by the mycotoxin OTA (Sabolić et al., 2007). Although this evidence does not explain the difference obtained between OTA urine concentrations of different genders, it suggests that further work is missing, for instance regarding the susceptibility of each gender in nephropathic condition presumably induced by or in which OTA might be involved, like BEN and/or CIN.

As for age, no study clearly correlates OTA urine contamination with age, although Gilbert et al. (2001) observed a lower mean level on the >45-year-old participants.

Although OTA in urine is a promising alternative, it is still waiting for further developments on the relationship at the individual level between OTA intake and the urinary biomarker (the mother compound or its metabolites), as well as the temporal variations in the presented levels. The latter is important for defining whether OTA in urine can be approached as a biomarker of chronic or acute exposure, and if it suffers from the same within-subject variability as plasma.

2.1.3.Human OTA milk biomarkers

Breastfeeding benefits have been well documented, for mothers and infants; nevertheless the newborn potential

risks of exposure, continuous or intermittent, to xenobiotics such as OTA may be relevant and requires a biological monitoring in general prevention (Jonsyn et al., 1995; Turconi et al., 2004). Galtier et al. (1977) suggested that OTA is transferred through the blood-milk barrier by passive diffusion in the nonionized form of the free toxin. Indeed the octanol-water partition ratio ($\log K_{ow}$) of OTA is 4.74 (García-Fonseca et al., 2008). However, modern studies on other epithelial barriers have shown the existence of an active transport (Ferrufino-Guardia et al., 2000). Recent evidence suggests that the excretion into human milk is mediated by breast cancer–related protein (BCRP), a member of the ATP-dependent efflux transporter family, which is highly expressed during lactation in various species, including humans, and with OTA as a substrate, which correlates with the high prevalence of this mycotoxin in human breast milk samples. These findings offer the possibility of estimating the likelihood of galactogenic excretion through rapid in vitro assays, which can be applied to all chemical classes of mycotoxins (Fink-Gremmels, 2008).

The galactogenic excretion serves to reduce maternal body burdens but signifies that breastfed infants are exposed to OTA (EFSA, 2006; JECFA, 2008). The implication that the mother's milk could be a potential source of OTA exposure to the offspring is aggravated by the fact that the maternal milk is the only food that most of the newborns consume in the first few months of life (Breitholtz-Emanuelsson et al., 1993a; Turconi et al., 2004). The assessment of OTA in milk permits the estimation of the degree of dietary exposure of infants to the toxin through milk (EI-Sayed et al., 2002) and may in addition be regarded as an indicator of exposure of a subgroup of the overall population, the lactating women (Jonsyn et al., 1995; Micco et al., 1995). If used with the latter purpose, it can stand as an alternative to the conventional blood and urine matrices, for two reasons: first for the easy collection during breastfeeding (the only drawback is to let the baby suckle before to diminish the mother's and suckling's anxiety); the contamination of the milk was reported as unrelated to smoking, age, parity, and anthropometric data other than body weight. Analysis of human milk samples may hence serve as an additional marker of exposure (EFSA, 2006).

The disadvantages of OTA milk biomarker lie in the fact that the levels are lower than the ones detected in blood. OTA plasma concentration was reported as 7 to 8 times higher than in rabbit milk (Galtier et al., 1977), and as 10 times higher in human milk (Breitholtz-Emanuelsson et al., 1993a). Although the concentration of OTA in milk is assumed to reflect the concentration of OTA in blood, the mechanism of OTA transport into milk and the distribution ratio between blood and human milk is not known. Variation in OTA levels in milk may be due to individual differences in bioavailability, distribution, and excretion of OTA (Skaug et al., 2001). On the other hand, the variation of the milk/plasma ratio values provided by the literature may reflect differences in the mode of exposure to the toxin (Ferrufino-Guardia et al., 2000). Studying the transport of OTA to the milk in female rabbits, Galtier et al. (1977) reported a milk/plasma concentration ratio of 0.1–0.2, after a single intravenous administration of OTA (1000–4000 mg/kg·bw). Breitholtz-Emanuelsson et al. (1993b) found a somewhat higher milk/plasma concentration ratio (0.2–0.5) in rats administered a single oral dose of OTA (10–250 mg/kg·bw). A significantly lower value (0.015) was obtained in rabbits after prolonged oral ingestion of contaminated feed (10–20 g/kg·bw/day). Nevertheless, a correlation between OTA concentration in maternal blood serum and milk has been confirmed and estimated at 0.0058 on average by Postupolski et al. (2006). Contrarily, in a previous study of Breitholtz et al. (1993a) no correlation between the OTA concentrations in milk and blood could be detected. The percentage of OTA detection in milk and correspondent blood samples were of 58% and 100%, respectively.

Another limitation of this biomarker is the fact that lactating women are not representative of the general population regarding eating and drinking habits, age, or smoking (Skaug et al., 2001). Furthermore, in the need of a whole-population monitoring survey, this matrix is not useful, since nonlactating individuals—men, children, elderly, and nonlactating women—are not represented.

The neonatal exposure to OTA is of concern due to the wide spectrum of harmful effects along with the lower body weight, higher metabolic rate, lower ability to detoxify, and incomplete development of some organs and tissues, notably the central nervous system of the newborns (Skaug et al., 2001; Navas et al., 2005). In fact, the milk is the major or even only food eaten by the rapidly growing young who are theoretically at their most vulnerable stage of development as far as induction of carcinogenesis and susceptibility to other toxic effects are concerned (EI-Sayed et al., 2002). The elucidation of the infant intake of contaminants from nursing (Micco et al., 1995) should therefore be of great importance and justifies a separate component of the risk assessments, which up until now do not differentiate between the risk to neonates/infants and the risk to adults.

Ferrufino-Guardia et al. (2000) found a linear relationship between the OTA concentrations in rabbit does milk and in the suckling's plasma, indicating an effective transfer of the toxin. If the same is true in humans, the exposure of the breastfed infant to the toxin, which has been largely reported in the literature, should be a major matter of concern for human health. In reality, the range of contamination levels in human milk provided by most studies (Table 4) are very close to the levels found in rabbit milk (49 ng/L) by Ferrufino-Guardia et al. (2000). However, a much higher concentration was obtained in Italy and Hungary (Micco et al., 1991, 1995; Kovacs et al., 1995) and extremely high levels were described in Sierra Leone (Jonsyn et al., 1995). As displayed in Table 4, the overall incidence and range of levels are markedly different between different countries, which might reflect the lower (Australia, Brazil) or higher (Sierra Leone, Italy, Egypt) incidence of OTA contamination of foodstuffs.

However, the comparison of incidences between different studies needs to be cautious, bearing in mind that higher detection limits will undoubtedly drop the percentage of positive samples.

The highest level was reported in a study conducted in Sierra Leone where 35% of samples contained OTA at levels up to 337 ng/ml, and so above the maximum permissible in animal feed and animal milk in some developed countries (Jonsyn et al., 1995). Although not even these are in the range that causes immediate morbidity, an intermittent or continuous exposure could predispose the kidneys to renal diseases early in life. In Africa, some common renal diseases in childhood remain poorly defined in relation to the etiologic nature, namely urinary tract infection (UTI), acute glomerula nephritis, and the quartan malarial nephrotic syndrome, for which the role of OTA should be scrutinized. With that purpose, through the analysis of both mothers' milk and infants' sera, Hassan et al. (2006) tried to address OTA's correlation with infants' kidney functions in Egypt. The results showed that the presence of OTA was associated with significantly higher levels of urinary β_2-microglobulin and microalbuminuria.

The observations and comparisons between milk concentrations and estimated daily intake should be considered approximate, since the pattern of OTA distribution in human milk is unknown. Indeed parameters such as fluctuations over the duration of breastfeeding and over the day, etc., can influence the composition of milk and consequently the release of OTA (Micco et al., 1995). Nevertheless, the proposed Nordic Working Group (NWG, 1991) tolerable daily intake (TDI) of 5 ng/kg of body weight was exceeded in the breastfed infant group studied by Skaug et al. (1998; 2001) and Turconi et al. (2004). Previously, in Italy, Micco et al. (1995) found a contamination level in milk that largely exceeded the tolerable weekly intake (PTWI) set by JECFA (1991), at that time at 112 ng/kg·bw, even if a safety factor of 2 or more is added, since it is an extrapolation of a health hazard from adults to infants. In Brazil, confirming a generally low incidence in commodities and hence a low population exposure to this toxin, Navas et al. (2005) provided an EDI for a 4-kg baby lower than the NWG (1991) TDI.

Since contaminated foodstuffs are the primary source of OTA exposure, its presence in human milk as a biomarker of the dietary exposure of the mother should be related to the dietary habits and intakes. Hence several studies attempted to examine the relationship or even to correlate the contamination of human milk and the dietary intake to trace the sources of OTA in milk. In Italy, Turconi et al. (2004) found a correlation between the potential risk factors and milk concentration only for bread consumption, probably due to the high intake frequency, more than twice a day. The largest consumers of milk, cheese, bread cereals, legumes, coffee, peanuts, pork meat, and cacao had higher mean values of OTA than the occasional consumers, but only a statistically significant difference emerged for bread. Also in Italy, Galvano et al. (2008) reported statistically significant higher OTA levels in milk of habitual consumers of bread, bakery products, and cured pork meat. No other statistically significant differences were observed, although habitual consumers of pasta, cookies, and juices had mean contamination values of OTA higher than the moderate consumer. The correlation between pork meat consumption and OTA in milk was further confirmed by Biasucci et al. (2010). Previously, in Norway, Skaug et al. (2001) showed that lactating women with a high dietary intake of liver paste (liverwurst, liver pâté) and cakes (cookies, fruitcakes, chocolate cakes, etc.) were more likely to have OTA-contaminated milk. The risk of OTA contamination was also increased by the intake of juice of all kinds. In addition, the results indicate that breakfast cereals, processed meat products, and cheese could be important contributors to dietary OTA intake. OTA contamination of the milk was unrelated to smoking, age, parity, and anthropometric data other than body weight. The donors with the most highly contaminated milk differed from those with less or not contaminated milk by having a significantly higher body weight. The authors also highlighted the trend towards increased prevalence and contamination level of OTA in milk samples collected during winter season (October–April) as compared with the summer season (May–September). Dietary and drinking habits vary between seasons, and between countries according to tradition and cultural differences. In addition, fluctuations in mould growth and contamination level of cereals may result in seasonal variations in dietary exposure to OTA (Skaug et al., 2001).

As for regional variations, the level of infant exposure to OTA from human milk reported in Norway by Skaug et al. (1998) presented significant variations between regions. The authors justified the geographical variations in the level of OTA contamination of the milk with differences in dietary habits, and also with regional differences in inhalation exposure to fungal spores. In the northern part of the country (north of the Arctic Circle), there was a significantly lower incidence and level of OTA contamination of the milk. There is only minor farming activity (grain processing, hay handling, etc.) in the northern areas, which might reduce the exposure to airborne fungal spores. In Egypt, 75% of the contaminated lactating women studied by Hassan et al. (2006) were from urban areas, contrarily to the assumption that a higher mycotoxin exposure was superior in rural areas, due to shoddier conditions of production and storage of cereals.

When comparing contamination incidence and OTA levels between hospitalized and nonhospitalized mothers, Micco et al. (1995) found that the milk of the first group had a higher incidence but a lower level of contamination. A possible explanation of such a discrepancy may lie in the different procedures of food preparation: in hospital, food is prepared in bulk, resulting in a more widespread OTA contamination but a lower contamination level. On the other hand, home prepared food accounts for a less frequent contamination of milk (16% versus 30%), but at a higher level. In this study no correlation could be established between OTA contamination and diet.

In summary, the results of the scarce studies of OTA concentration in milk point out the need to continue biological monitoring (Turconi et al., 2004) and to conduct risk assessments for OTA that differentiate between the risk to children and the risk to adults (Skaug et al., 1998).

2.1.4.Other biomarkers

Hardly any studies exist on the presence of OTA in semen. Pilot experiments indicated that OTA ingested at 5 and 10 times the human tolerable daily intake (TDI = 16 ng OTA/kg·bw per day; JECFA, 1991) could be detected in the seminal plasma of boars, and toxin profiles correlated to serum profiles (Solti et al., 1999). In toxicokinetic studies, OTA was found in testicles of laboratory animals (Gharbi et al., 1993). Furthermore, several effects of OTA on the male reproductive system were reported, specifically testosterone secretion inhibition in isolated testicular interstitial cells of gerbils (Fenske and Fink-Gremmels, 1990), disturbance of spermatogenesis (Gharbi et al., 1993), and decreased mobility and life time of spermatozoids (Solti et al., 1999). Furthermore, some authors hypothesize about the relation between OTA-contaminated food consumption and human testicular cancer (Schwartz, 2002). In a more recent study, Biró et al. (2003) challenged boars through OTA ingestion to investigate the correlation between the serum and seminal fluid toxin levels. Following OTA exposure, the toxin profile of seminal plasma was different from that of serum and showed a significant delay in time to reach maximal concentration. The average OTA level remained considerably lower compared to serum, representing about one fifth of the serum values throughout the whole toxin application period. Maximal mean concentration (0.402 ± 0.0277 ng/ml) was reached by the end of toxin administration only, i.e., 4 weeks later than in serum. Contrary to the plasma profile, no negative exponential phase was noticed. During the simultaneous histopathological examination, a small number of altered cell structures was observed, which was regarded as hormonal or mechanical effects, and not a direct toxic effect. The authors did not, however, exclude the possibility that the excreted OTA in the seminal plasma might affect the spermatozoa viability, forward motility, and longevity. This effect on sperm production and boar semen quality occurs only after a lag period.

Feces analysis of challenged sheep by Höhler et al. (1999) reflected the intake of OTA, as did serum and urine. In the three studied biological matrices, OTA and OTα were found in significant concentrations, indicating a substantial escape of OTA from ruminal and hindgut fermentation. The percentage of OTA and OTα that was excreted was relatively high (over 75% of the total ingested OTA). However, relatively small amounts of intact OTA were excreted in the urine and feces, as expressed to the total intake, 3.6–3.8% and 0.5–0.8%, respectively. In reality, the majority of the excreted OTA in urine was OTα (60–70%), in contrast to the 10% of the same metabolite found in feces. In the stool specimens analyzed from <5-year-old infants from Sierra Leone (Jonsyn-Ellis, 1999), the metabolite detected was 4R-OTA, at a contamination frequency of 46% (ranging from 0.02 to 2.5 ng/ml), very similar to the therein reported value in urine of 45%. The intact OTA was detected in 33% of the stool feces (ranging from 0.1 to 14 ng/ml).

2.2.Biomarkers of effective dose

Biomarkers of effective dose indicate that exposure to a particular compound has resulted in the compound or its metabolite(s) reaching a toxicologically significant target. Because of the many possible interindividual differences in the rate and route of metabolism of compounds, the effective dose at the target site is a preferred measurement over the internal dose (Timbrell, 1998). This happens because the former reflects not only individual differences in absorption and distribution but also differences in the metabolism (activation versus detoxification) and the extent of repair of DNA damage (Pfohl-Leszkowicz et al., 2007).

The effective dose is often determined by measuring specific adducts in tissues or body fluids. Chemicals that are reactive (electrophilic) or are metabolized to reactive intermediates (electrophiles) that react with DNA are of particular interest and concern in relation to genotoxicity, and therefore possible carcinogenicity. The presence of a chemical-specific DNA adduct in human DNA is a good indication that exposure to that chemical occurred, and so are used as exposure biomarkers (Timbrell, 1998; Pfohl-Leszkowicz et al., 2007).

The concentration of adducts measured in a DNA sample is the result of a balance between rates of formation, removal by repair and cell death, and dilution by DNA replication as the newly synthesized strands do not contain adducts. The life time of DNA adducts depends on their structure (i.e., the genotoxic from which they are derived) and generally it lies in the range of days to weeks (occasionally a few months) (Kyrtopoulos, 2006). DNA adducts are believed to be markers of exposure over such a period, and hence they generally reflect recent exposure rather that in the distant past. A sporadic DNA adduct measurement is, therefore, somewhat noninformative if not related to consideration of the timing and duration of exposure (Poirier et al., 2000).

The weight of evidence supports the notion that exposure to most chemical carcinogens results in damage to the structural integrity of DNA, which occurs primarily as covalent carcinogen binding and is referred to as carcinogen-DNA adduct formation (Poirier et al., 2000). But in the specific case of OTA, there is no agreement on the formation of these OTA-related DNA adducts and conflicting results have been reported. Some studies support the existence of OTA-DNA adducts (Obrecht-Pflumio and Dirheimer, 2000; Castegnaro et al., 2006; Mantle et al., 2010), and use them for instance to provide evidence of the implication of OTA in BEN and associated UTT patients (Pfohl-Leszkowicz et al., 2007); whereas several other publications have alleged the opposite (Schlatter et al., 1996; Gautier et al., 2001; Gross-Steinmeyer et al., 2002; Mally et al., 2004) and reject its relevance to a genotoxic mode of action for OTA, in favor of oxidative stress.

It was mainly ^{32}P-postlabeling assays that showed that OTA treatment induced a dose- and time-dependent formation of DNA adducts in different organs of mice and rat, which persisted up to 16 days in kidneys (Pfohl-Leszkowicz et al., 1993). Using the same postlabeling method, OTA-DNA adducts were also reported in in vitro experiments, namely in cell cultures (Grosse et al., 1995, 1997) and with rabbit liver and kidney microsomes treated with OTA (Obrecht-Pflumio and Dirheimer, 2000).

Evidences for interaction of OTA with DNA were further reported following chronic exposure to rat and subacute exposure to pig, demonstrating, according to the authors, the potential of the OTA phenoxyl radical to form covalent C8-deoxyguanosine (C8-dG) adducts in vivo (Faucet et al., 2004).

For the suspected role of OTA in BEN, Pfohl-Leszkowicz et al. (2007) studied DNA adduct formation and persistence in human kidney cells treated with OTA. The authors reported the formation of large amounts of OTA-DNA adducts, which disappeared after 2 days. The authors also found DNA adducts related to OTA in human kidney tumors from patients from BEN region, several of which featuring the same chromatographic properties than OTA hydroxyquinone (OTHQ)-DNA and C-C8-dG-OTA-DNA adducts. The formation of either OTHQ- or C-C8-dG-OTA-DNA adduct was previously reported (Tozlovanu et al., 2006) as depending of the expression of some biotransforming enzymes. The first was formed after in vitro incubation in presence of kidney microsomes of untreated pig or healthy human expressing mainly cyclooxygenase 1 (COX1) and cytochrome P450 2C9 (CYP2C9), whereas C-C8-dG-OTA was formed mainly after incubation in presence of kidney microsomes from pig fed OTA or from human tumor, expressing mainly COX2 and lipoxygenase (Tozlovanu et al., 2006).

Jennings-Gee et al. (2010) reported, for the first time, the formation of OTA-DNA adducts after maternal exposure. These adducts, detected in the testes of newborn mice after intrauterine exposure to OTA, were chromatographically similar to adducts in kidney and in testicular DNA that were produced by feeding OTA to adult mice by gavage (i.e., via the diet). The authors further suggest the hypothesis that OTA may play a role in the etiology of testicular cancer. The main adduct observed in the testis co-migrated with the C-C8-dG-OTA adduct, which was also the main adduct in the kidney (Jennings-Gee et al., 2010). The same C-C8-dG-OTA adduct was afterwards identified by tandem mass spectroscopy (MS/MS) (Mantle et al., 2010).

Based on the formation of a C8-dG adduct through a phenolic radical of OTA, Dai et al. (2003) have earlier proposed a mechanism of genotoxicity for phenolic toxins: formation of reactive oxygen species (ROS) with subsequent oxidative damage and coupling of reactive phenolic radical intermediates to the C8 position of guanosine to form DNA adducts. In another hypothesis, the same authors further suggest the possibility of the phenolic radical cause the oxidation of glutathione into a thiyl radical, which would lead to the formation of glutathione disulfide and a ROS, the superoxide anion radical $O_2^{\bullet -}$.

By contrast, Mally et al. (2004), using the highly sensitive accelerator mass spectrometry technique, did not detect DNA adducts after the administration of ^{14}C-labeled OTA to rats. Other studies with radiolabeled OTA were also unable to detect any DNA binding of OTA (Gautier et al., 2001; Gross-Stein-Meyer et al., 2002; Delatour et al., 2008). Thus one major factor of debate is that the formation of OTA-DNA adducts was mostly proven through ^{32}P-postlabeling assays, considered as unspecific and unable to identify or characterize the chemical structure; furthermore, this technique is regarded as unsuitable to eliminate the possible role of lipid peroxidation, and hence clarify if these are formed through an indirect mechanism or are actually related to OTA itself (EFSA, 2006; JECFA, 2008).

Additionally, as stated before, DNA adducts are frequently used as biomarkers of exposure to chemicals that are either electrophilic or are metabolized to electrophiles (Pfohl-Leszkowicz et al., 2007). According to one of the suggested hypothesis, as reviewed by Manderville and Pfohl-Leszkowicz (2008), OTA forms a benzoquinone electrophile following activation by cytochrome P450 enzymes and radical species following activation of enzymes with peroxidase activities. These electrophiles react preferentially with deoxyguanine to form benzetheno adduct and C8-dG-OTA. Analysis of OTA-mediated DNA adduct by ^{32}P-postlabeling method correlates with OTA chemistry and adduct spots derived from quinone electrophiles that are generated following activation by cytochrome P450, whereas a C8-dG-OTA adduct is formed following activation of OTA by peroxidases, mainly contained in kidney and testis. Nevertheless, Mosesso et al. (2008) demonstrated that OTA does not bear any clastogenic or aneugenic activity based on the absence of the induction of chromosome aberrations, sister-chromatid exchanges, and micronuclei in human lymphocytes and V79 cells in vitro. Since structural chromosome aberrations are a very sensitive endpoint to detect chemical carcinogens with electrophilic substituents, the authors assume that OTA or its possible metabolites do not covalently bind DNA through the formation of adducts.

According to the EFSA scientific panel on contaminants in the food chain, at the time of the most recent evaluation (EFSA, 2006), and after considering all the available data, there is no evidence for the existence of specific OTA-DNA adducts and that the genotoxic effects of OTA were most likely attributed to oxidative stress. According to Mally and Dekant (2009), in a review of the proposed modes of action for OTA kidney tumorigenicity, the absence of covalent DNA adducts is consistent with the lack of bioactivation to reactive metabolites and the finding that OTA-mediated genotoxicity in mammalian cells is independent of metabolic activation.

3. Biomarkers of effect

Biomarkers of effect or response can be broadly divided into invasive and noninvasive and those that indicate pathological damage and those that detect biochemical changes or responses (Timbrell, 1998). Most of the ones described for OTA are unspecific, the most studied one being β_2-microglobulin (β2M). This low-molecular-weight (11.8-kDa) protein is the light chain of the major histocompatibility (MHC) class I molecule expressed on the cell surface of all nucleated cells. β2M dissociates from the heavy chain in the setting of cellular turnover and enters the circulation as a monomer. β2M is typically filtered by the glomerulus and almost entirely reabsorbed and catabolized by the proximal tubular cells, a process that may be impeded in kidney injury. Increased urinary β2M excretion has been regarded as an early marker of tubular injury in a number of settings, including nephrotoxicant exposure. Despite being a sensitive indicator of tubular dysfunction and for that reason used as an index of renal tubular disorders, it is considered nonspecific, since it is expressed on the surface of all nucleated cells. Furthermore, its utility as a biomarker has been limited by its instability in urine, with rapid degradation observed at room temperature and in urine with a pH of less than 6.0 (Vaidya et al., 2008).

Several studies show that β_2-microglobulinuria seems to be associated with nephropathy such as BEN and CIN. There are reports on increased β2M excretion in patients with BEN (Hall et al., 1973), their clinically healthy relatives (Stefanovic et al., 1991), adult offspring of families with BEN (Dimitrov et al., 2006), as well as in children from these families (Stefanovic et al., 2003). In patients with BEN, their healthy relatives, and children from BEN families, urinary albumin excretion was also found increased (Stefanovic et al., 2002). Raised urinary β2M levels have been reported in association with impairment of renal tubular function, correlating with high levels of OTA exposure in patients with CIN syndrome living in an area of Tunisia known to have high dietary exposure to OTA (Hassen et al., 2004). Kidney function in infants' breastfed for at least 4 months has been assessed in relation to maternal and infant serum and maternal milk concentrations of OTA. Infants with higher OTA levels in their serum (≥2 ng/ml) had higher levels of urinary β2M and microalbuminuria than did infants with lower serum levels (<2 ng/ml). Both differences were statistically significant with the use of univariate analysis. However, multivariate logistic regression analysis showed that there was a significant correlation between a higher infant serum level of OTA and the degree of microalbuminuria, but not β_2-microglobulinuria (Hassan et al., 2006). Microalbuminuria (defined as the pathologic excretion of urinary albumin at levels of 30 to 300 μg/ml) has long been established as a useful marker of the development and progression of renal disease. Attention should, however, be paid to the fact that it may also be caused by vigorous exercise, hematuria, urinary tract infection, and dehydration (Vaidya et al., 2008).

Although proteinuria and leakage of tubular enzymes have been described in controlled experiments with OTA, these parameters cannot be used in epidemiological investigation, as similar changes can be induced by a multiplicity of other nephrotoxic compounds, including commonly used drugs such as nonsteroidal anti-inflammatory drugs (EFSA, 2006)

Accordingly, analysis of urine samples for markers of proximal tubule damage associated with exposure to OTA is considered to be nonspecific.

4. Conclusion

After the review of the available data and the discussion of the usefulness and limitations of the currently available and most used OTA exposure biomarkers, it is noticeable a lack of information on toxicokinetics of the mycotoxin, which hinders further evaluation and confirmation of the accuracy and reliability of the OTA biomarkers. The same is true for the few described OTA effect biomarkers. Nevertheless, and despite the drawbacks, implementation of different biomarkers allowed a confirmation of a broad exposure to OTA, and so echoing the several previous and ongoing surveys that also demonstrate a similarly broad contamination of foodstuffs, believed to be the main source of exposure.

It is important to underline, however, that direct measurements of exposure through external dose and measurements of internal dose are not substitutes for each other. They are complementary rather than competing methods for conducting realistic exposure assessments and will undoubtedly strengthen exposure assessments. As this field continues to expand, additional biospecimen types and putative biomarkers may be investigated along with the influence of individual, seasonal, or geographical features on the ones already used. It is also envisaged that biomarkers of the effects of mycotoxin exposures will follow from this development. For that purpose, further investigation needs to be conducted namely in the field of toxicokinetics.

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Declaration of interest

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References

Abid S, Hassen W, Achour A, Skhiri H, Maaroufi K, Ellouz F, Creppy E, Bacha H. (2003). Ochratoxin A and human chronic nephropathy in Tunisia: Is the situation endemic? Hum Exp Toxicol 22:77–84.

Abouzied MM, Horvath AD, Podlesny PM, Regina NP, Metodiev VD, Kamenova-Tozeva RM, Niagolova ND, Stein AD, Petropoulos EA, Ganev VS. (2002). Ochratoxin A concentrations in food and feed from a region with Balkan endemic nephropathy. Food Addit Contam 19:755–764.

Apostolou E, El-Nezami HS, Ahokas JT, Donohue DC. (1998). The evaluation of ochratoxin A in human milk in Victoria (Australia). Rev Méd Vét 149:709–711.

Aslam M, Beg AE, Blaszkewicz M, Degen GH, Golka K. (2005). Ochratoxin A blood concentration in healthy subjects and bladder cancer cases from Pakistan. Mycot Res 21:164–167.

Assaf H, Betbeder A-M, Creppy EE, Pallardy M, Azouri H. (2004). Ochratoxin A levels in human plasma and foods in Lebanon. Hum Exp Toxicol 23:495–501.

Bacha H, Maaroufi K, Achour A, Hamammi M, Ellouz F, Creppy EE. (1993). Ochratoxines et ochratoxicoses humaines en Tunisie. In: Creppy E, Castegnaro M, Dirheimer G, eds. Human Ochratoxicosis and Its Pathologies. Volume 231. Paris: John Libbey Eurotext, 123–127.

Bacha H, Maaroufi K, Ghedira-Chekir L, Abid S, Cherif A, Achour A, Creppy EE. (1999). Mycotoxins and mycotoxicosis in Tunisia: What do we know and what do we need to know? J Toxicol Toxin Rev 18:245–262.

Biasucci G, Calabrese G, Di Giuseppe R, Carrara G, Colombo F, Mandelli B, Maj M, Bertuzzi T, Pietri A, Rossi F. (In press). The presence of ochratoxin A in cord serum and in human milk and its correspondence with maternal dietary habits. Eur J Nutr DOI 10.1007/s00394-010-0130-y.

Biró K, Barna-Vetró I, Pécsi T, Szabó E, Winkler G, Fink-Gremmels J, Solti L. (2003). Evaluation of spermatological parameters in ochratoxin A-challenged boars. Theriogenology 60:199–207.

Breitholtz A, Olsen M, Dahlbäck Å, Hult K. (1991). Plasma ochratoxin A levels in three Swedish populations surveyed using an ion-pair HPLC technique. Food Addit Contam 8:183–192.

Breitholtz-Emanuelsson A, Olsen M, Oskarsson A, Palminger I, Hult K. (1993a). Ochratoxin A in cow's milk and in human milk with corresponding human blood samples. JAOAC Int 76:842–846.

Breitholtz-Emanuelsson A, Minervini F, Hult K, Visconti A. (1994). Ochratoxin A in human serum samples collected in southern Italy from healthy individuals and individuals suffering from different kidney disorders. Nat Toxins 2:366–370.

Breitholtz-Emanuelsson A, Palminger-Hallen I, Wohlin PO, Oskarsson A, Hult K, Olsen M. (1993b). Transfer of ochratoxin A from lactating rats to their offspring: A short-term study. Nat Toxins 1:347–352.

Bunge I, Dirheimer G, Röschenthaler R. (1978). In vivo in vitro inhibition of protein synthesis in Bacillus stearothermophilus by ochratoxin A. Biochem Biophys Res Commun 83:398–405.

Castegnaro M, Canadas D, Vrabcheva T, Petkova-Bocharova T, Chernozemsky IN, Pfohl-Leszkowicz A. (2006). Balkan endemic nephropathy: Role of ochratoxins A through biomarkers. Mol Nutr Food Res 50:519–529. 1

Castegnaro M, Maru V, Maru G, Ruiz-Lopez MD. (1990). High performance liquid chromatography determination of ochratoxin A and its 4R-4-hydroxy metabolite in human urine. Analyst 115:129–131.

Conseil Supérieur d'Hygiène Publique de France. (1999). Les mycotoxines dans l'alimentation: évaluation et gestion du risqué. Paris, France: Lavoisier, Tec & Doc.

Coronel MB, Sanchis V, Ramos AJ, Marin S. (2009). Assessment of the exposure to ochratoxin A in the province of Lleida, Spain. Food Chem Toxicol 47:2847–2852.

Creppy EE, Baudrimont I, Betbeder A-M. (1995). Prevention of nephrotoxicity of ochratoxin A, a food contaminant. Toxicol Lett 82–83:869–877.

Creppy EE, Betbeder AM, Gharbi A, Counord J, Castegnaro M, Bartsch H, Moncharmont P, Fouillet B, Chambon P, Dirheimer G. (1991). Human ochratoxicosis in France. IARC Sci Publ (115):145–151.

Curtui VG, Gareis M. (2001). A simple HPLC method for the determination of the mycotoxins ochratoxin A and B in blood serum of swine. Food Addit Contam 18:635–643.

Dahlmann A, Dantzler WH, Silbernagl S, Gekle M. (1998). Detailed mapping of ochratoxin A reabsorption along the rat nephron in vivo: The nephrotoxin can be reabsorbed in all nephron segments by different mechanisms. J Pharmacol Exp Ther:286:157–162.

Dai J, Wright MW, Manderville RA. (2003). Ochratoxin A forms a carbon-bonded C8-deoxyguanosine nucleoside adduct: Implications for C8 reactivity by a phenolic radical. J Am Chem Soc 125:3716–3717.

Delatour T, Mally A, Richoz J, Özden S, Dekant W, Ihmels H, Otto D, Gasparutto D, Marin-Kuan M, Schilter B, Cavin C. (2008). Absence of 29-deoxyguanosine-carbon 8-bound ochratoxin A adduct in rat kidney DNA monitored by isotope dilution LC-MS/MS. Mol Nutr Food Res 52:472–482.

Dimitrov P, Tsolova S, Georgieva R, Bozhilova D, Simeonov V, Bonev A, Karmaus W. (2006). Clinical markers in adult offspring of families with and without Balkan endemic nephropathy. Kidney Int 69:723–729.

Dinis AMP, Lino CM, Pena AS. (2007). Ochratoxin A in nephropathic patients from two cities of central zone in Portugal. J Pharm Biomed Anal 44:553–557.

Doerr JA, Huff WE, Tung HT, Wyatt RD, Hamilton PB. (1974). A survey of T-2 toxin, ochratoxin, and aflatoxin for their effects on the coagulation of blood in young broiler chickens. Poult Sci 53:1728–1734.

Domijan AM, Peraica M, Fuchs R, Lucić A, Radić B, Balija M, Bosanac I, Grgičević D. (1999). Ochratoxin A in blood of healthy population in Zagreb. Arh hig Rada Toksikol 50:263–271.

Domijan A-M, Peraica M, Markov K, Fuchs R. (2009). Urine Ochratoxin A and Sphinganine/Sphingosine ratio in residents of the endemic nephropathy area in Croatia. Arh Hig Rada Toksikol 60:387–393.

Dostal A, Jakusova L, Cajdova j, Hudeckova H. (2008). Results of the first studies of occurrence of Ochratoxin A in human milk in Slovakia. Bratisl Lek Listy 109:276–278.

Duarte S, Bento J, Pena A, Lino CM, Delerue-Matos C, Oliva-Teles T, Morais S, Correia M, Oliveira MBPP, Alves MR, Pereira JA. (2010). Monitoring of ochratoxin A exposure of the Portuguese population through a nationwide urine survey—Winter 2007. Sci Tot Environ 408:1195–1198.

Duarte SC, Bento JMV, Pena A, Lino CM. (2009a). Ochratoxin A exposure assessment of the inhabitants of Lisbon during winter 2007/2008 through bread and urine analysis. Food Addit Contam 26:1411–1420.

Duarte SC, Pena A, Lino CM. (2009b). Ochratoxin A non-conventional exposure sources—A review. Microchem J 93:115–120.

EFSA. (2006). Question no. EFSA.Q-2005-154. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food. EFSA.J 365:1–56.

El-Sayed AA, Neamat-Allah AA, Soher EA. (2000). Situation of mycotoxins in milk, dairy products and human milk in Egypt. Mycot Res 16:91–100.

EI-Sayed AA, Soher EA, Neamat-Allah AA. (2002). Human exposure to mycotoxins in Egypt. Mycot Res 18:23–30.

Erkekoğlu P, Sabuncuoğlu S, Aydın S, Şahin G, Giray B. (2010). Determination of seasonal variations in serum ochratoxin A levels in healthy population living in some regions of Turkey by enzyme-linked immunosorbent assay. Toxicon 55:507–513.

Faucet V, Pfohl-Leszkowicz A, Dai J, Castegnaro M, Manderville RA (2004). Evidence for covalent DNA adduction by ochratoxin A following chronic exposure to rat and subacute exposure to pig. Chem Res Toxicol 17:1289–1296.

Fazecas B, Tar A, Kovacs M. (2005). Ochratoxin a content of urine samples of healthy humans in Hungary. Acta Vet Hung 53:35–44.

Fenske M, Fink-Gremmels J. (1990). Effects of fungal metabolites on testosterone secretion in vitro. Arch Toxicol 64:72–75.

Ferrufino-Guardia EV, Tangni EK, Larondelle Y, Ponchaut S. (2000). Transfer of ochratoxin A during lactation: Exposure of suckling via the milk of rabbit does fed a naturally contaminated Feed. Food Addit Contam 17:167–175.

Filali A, Betbeder AM, Baudrimont I, Benayada A, Soulaymani R, Creppy EE. (2002). Ochratoxin A in human plasma in Morocco: A preliminary survey. Hum Exp Toxicol 21:241–245.

Fink-Gremmels J. (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: A review. Food Addit Contam Part A 25:172–180.

Galtier P, Baradat C, Alvinere M. (1977). Etude de l'élimination d'ochratoxine A par le lait chez la lapine. Ann Rech Vét 7:97–98.

Galvano F, Pietri A, Bertuzzi T, Gagliardi L, Ciotti S, Luisi S, Bognanno M, Fauci1 L, Iacopino AM, Nigro F, Volti GL, Vanella L, Giammanco G, Tina GL, Gazzolo D. (2008). Maternal dietary habits and mycotoxin occurrence in human mature milk. Mol Nutr Food Res 52:496–501.

García-Fonseca S, Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. (2008). Coacervative extraction of Ochratoxin A in wines prior to liquid chromatography/fluorescence determination. Anal Chim Acta 617:3–10

Gautier J-C, Richoz J, Welti DH, Markovic J, Gremaud E, Guengerich FP, Turesky RJ. (2001). Metabolism of ochratoxin A: Absence of formation of genotoxic derivatives by human and rat enzymes. Chem Res Toxicol 14:34–45.

Gekle M, Sauvant C, Schwerdt G. (2005). Ochratoxin A at nanomolar concentrations: A signal modulator in renal cells. Mol Nutr Food Res 49:118–130.

Ghali R, Hmaissia-Khlifa K, Mezigh CH, Ghorbel H, Maaroufi K, Maschgoul S, Hedilli A. (2008). HPLC Determination of ochratoxin A in a low volume of human blood serum. Anal Lett 41:757–766.

Gharbi A, Trillon O, Betbeder AM, Counord J, Gauret MF, Pfohl-Leszkowicz A, Dirheimer G, Creppy EE. (1993). Some effects of ochratoxin A, a mycotoxin contaminating feeds and food, on rat testis. Toxicology 83:9–18.

Gilbert J, Brereton P, MacDonald S. (2001). Assessment of dietary exposure to ochratoxin A in the UK using a duplicate diet approach and analysis of urine and plasma samples. Food Addit Contam 18:1088–1093.

Grosse Y, Baudrimont I, Castegnaro M, Creppy EE, Dirheimer G, Pfohl-Leskowicz A. (1995). Ochratoxin A metabolites and DNA-adducts formation in monkey kidney cell. Chem Biol Interact 95:175–187.

Grosse Y, Chekir-Ghedira L Huc A, Obrecht-Pflumio S, Dirheimer G, Bacha H, Pfohl-Leskowicz A. (1997). Retinol ascorbic acid and alpha-tocopherol prevent DNA adduct formation in mice treated with the mycotoxin ochartoxin A and zearalenone. Cancer Lett 114:225–229.

Grosso F, Saïd S, Mabrouk I, Fremy JM, Castegnaro M, Jemmali M, Dragacci S. (2003). New data on the occurrence of ochratoxin A in human sera from patients affected or not by renal diseases in Tunisia. Food Chem Toxicol 41:1133–1140.

Gross-Steinmeyer K, Weymann J, Hege HG, Metzler M. (2002). Metabolism and lack of DNA reactivity of the mycotoxin ochratoxin a in cultured rat and human primary hepatocytes. J Agric Food Chem 50:938–945.

Gürbay A, Girgin G, Sabuncuoğlu SA, Şahin G, Yurdakök M, Yiğit Ş, Tekinalp G. (2009). Ochratoxin A: Is it present in human breast milk samples obtained mothers from Ankara, Turkey. In: Abstracts of the 46th Congress of the European Societies of Toxicology; 13–16 September 2009; Dresden, Germany. Toxicol Lett 189(Supp 1):S57–S273.

Gupta M, Bandopadhyay S, Paul B, Majumder SK. (1979). Hematological changes produced in mice by ochratoxin A. Toxicology 14:95–98.

Hagelberg S, Hult K, Fuchs R. (1989). Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. J Appl Toxicol 9:91–96.

Hall PW, Vasiljević M. (1973). Beta2-microglobulin excretion as an index of renal tubular disorders with special reference to endemic Balkan nephropathy. J Lab Clin Med 81:897–904.

Hassan AM, Sheashaa HA, Fattah MFA, Ibrahim AZ, Gaber OA, Sobh MA. (2006). Study of ochratoxin A as an environmental risk that causes renal injury in breast-fed Egyptian infants. Pediatr Nephrol 21:102–105.

Hassen W, Abid S, Achour A, Creppy E, Bacha H. (2004). Ochratoxin A and β_2-microglobulinuria in healthy individuals and in chronic interstitial nephropathy patients in the Centre of Tunisia: A hot spot of Ochratoxin A exposure. Toxicology 199:185–193.

Höhler D, Sudekum KH, Wolffram S, Frohlich AA, Marquardt RR. (1999). Metabolism and excretion of ochratoxin A fed to sheep. J Anim Sci 77:1217–1223.

Hult K, Plestina R, Habazin-Novak V, Radic B, Ceovic S. (1982). Ochratoxin A in human blood and Balkan endemic nephropathy. Arch Toxicol 51:313–321.

IARC. (1993). Some Naturally Occurring Substances, Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Monograph no. 56. Lyon, France: IARC.

Iavicoli I, Brera C, Carelli G, Caputi R, Marinaccio A, Miraglia M. (2002). External and internal dose in subjects occupationally exposed to ochratoxin A. Int Arch Occup Environ Health 75: 381–386.

JECFA. (1991). Evaluation of Certain Food Additives and Contaminants. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 806. Geneva: Joint FAO/WHO Expert Committee on Food Additives

JECFA. (2001). Safety Evaluation of Certain Mycotoxins in Food. WHO Food Additives Series 47; FAO Food and Nutrition Paper 74. Available at: http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm. Accessed 10 August 2008.

JECFA. (2007). Safety Evaluations of Specific Mycotoxins: Ochratoxin A. Prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, 6–15 February, Geneva.

JECFA. (2008). Safety Evaluation of Certain Mycotoxins in Food. WHO Food Additives Series 59. Available at: http://whqlibdoc.who.int/publications/2008/978924 1660594_eng.pdf. Accessed 18 November 2009.

Jennings-Gee JE, Tozlovanu M, Manderville R, Miller MS, Pfohl-Leszkowicz A, Schwartz GG. (2010). Ochratoxin A: In utero exposure in mice induces adducts in testicular DNA. Toxins 2:1428–1444.

Jiménez AM, Lopez de Cerain˜ A, Gonzalez-Peñas E, Bello J, Betbeder AM, Creppy EE. (1998). Exposure to ochratoxin A in Europe: Comparison with a region of northern Spain. Toxin Rev 17:479–491.

Jonsyn FE, Maxwell SM, Hendrickse RG. (1995). Ochratoxin A and aflatoxins in breast milk samples from Sierra Leone. Mycopathology 131:121–126.

Jonsyn FE. (1999). Intake of aflatoxins and ochratoxins by infants in Sierra Leone: Possible effects on the general health of these children. J Nutr Environ Med 9:15–22.

Jonsyn-Ellis FE. (2000). Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. Mycopathology 152:35–40.

Jonsyn-Ellis FE. (2007). Aflatoxins and ochratoxins in serum samples of school children. J Nutr Environ Med 16:52–58.

Jørgensen K, Petersen A. (2002). Content of ochratoxin A in paired kidney and meat samples from healthy Danish slaughter pigs. Food Addit Contam 19:562–567.

Karima H-K, Ridha G, Zied A, Chekib M, Salem M, Abderrazek H. (2010). Estimation of Ochratoxin A in human blood of healthy Tunisian population. Exp Toxicol Pathol 62:539–542.

Khalef A, Zidane C, Charef A, Gharbi A, Tadjerouna M, Betbeder AM, Creppy EE. (1993). Ochratoxicoses humaines en Algérie. In: Creppy E, Castegnaro M, Dirheimer G, eds. Human Ochratoxicosis and Its Pathologies. Volume 231. Paris: John Libbey Eurotext, 123–127.

Kovács F, Sándor G, Ványi A, Domány S, Zomborszky-Kovács M. (1995). Detection of ochratoxin A in human blood and colostrum. Acta Vet Hung 43:393–400

Krogh P, Hald B, Plestina R, Ceović S. (1977). Balkan (endemic) nephropathy and foodborne Ochratoxin A: Preliminary results of a survey of foodstuffs. Acta Pathol Microbiol Scand B 85, 238–240.

Krogh P. (1977). Ochratoxin A residues in tissues of slaughter pigs with nephropathy. Nord Vet Med 29:402–405.

Kuiper-Goodman T, Hilts C, Billiard SM, Kiparissis Y, Richard IDK, Hayward S. (2010). Health risk assessment of ochratoxin A for all age-sex strata in a market Economy. Food Addit Contam 27, 212–240.

Kuiper-Goodman T. (1990). Uncertainties in the risk assessment of three mycotoxins: Aflatoxin, ochratoxin, and zearalenone. Can J Physiol Pharmacol 68:1017–1024.

Kyrtopoulos SA. (2006). Biomarkers in environmental carcinogenesis research: Striving for a new momentum. Toxicol Lett 162:3–15.

Larsen JC. (1995). Levels of pollutants and their metabolites: Exposures to organic substances. Toxicology 101:11–27.

Li S, Marquardt RR, Frohlich AA. (2000). Identification of ochratoxins and some of their metabolites in bile and urine of rats. Food Chem Toxicol 38:141–152.

Lino CM, Baeta ML, Henri M, Dinis AMP, Pena AS, Silveira MIN. (2008). Levels of ochratoxin A in serum from urban and rural Portuguese populations and estimation of exposure degree. Food Chem Toxicol 46:879–885.

Maaroufi K, Achour A, Hamammi M, El May M, Betbeder AM, Ellouz F, Creppy EE, Bacha H. (1995). Ochratoxin A in human blood in relation to nephropathy in Tunisia. Hum Exp Toxicol 14:609–615.

MAFF—Ministry of Agriculture, Fisheries and Food. (April 1999). A Survey of Human Exposure to Ochratoxin A. Food Surveillance Information Sheet, number 172. London, United Kingdom.

Mally A, Dekant W. (2009). Mycotoxins and the kidney: Modes of action for renal tumor formation by ochratoxin A in rodents. Mol Nutr Food Res 53:467–478.

Mally A, Hard GC, Dekant W. (2007). Ochratoxin A as a potential etiologic factor in endemic nephropathy: Lessons learned from toxicity studies in rats. Food Chem Toxicol 45:254–2260.

Mally A, Zepnik H, Wanek P, Eder E, Dingley K, Ihmels H, Völkel W, Dekant W. (2004). Ochratoxin A: Lack of formation of covalent DNA adducts. Chem Res Toxicol 17:234–242.

Manderville RA, Pfohl-Leszkowicz A. (2008). Bioactivation and DNA adduction as a rationale for ochratoxin A carcinogenesis. World Mycot J 1:357–367.

Manique R, Pena A, Lino CM, Moltó JC, Mañez J. (2008). OchratoxinA in themorning and afternoon portions of urine from Coimbra and Valencia populations. Toxicon 51:1281–1287.

Mantle PG, Faucet-Marquis V, Manderville RA, Sciqualli B, Pfohl-Leszkowicz A. (2010). Structures of covalent adducts between DNA and ochratoxin A: A new factor in debate about genotoxicity and human risk assessment. Chem Res Toxicol 23:89–98.

Micco C, Ambruzzi A, Miraglia M, Brera C, Onori R, Benelli L. (1991). Contamination of human milk with ochratoxin A. In: Castegnaro PR, Plestina R, Dirheimer M, Chernozemsky G, Bartsch IN, eds. Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. Lyon, France: IARC, 105–108.

Micco C, Miraglia M, Brera C, Corneli S, Ambruzzi A. (1995). Evaluation of ochratoxin A level in human milk in Italy. Food Addit Contam Part A 12:351–354.

Miraglia M, Brera C, Colatosti M. (1996). Application of Biomarkers to assessment of risk to human health from exposure to mycotoxins. Microchem J 54:472–477.

Miraglia M, Brera C. (2002). Assessment of dietary intake of ochratoxin A by the population of EU member states. SCOOP/2002. Task 3.2.7. Brussels: European Union.

Mosesso P, Cinelli S, Piñerro J, Bellacima R, Pepe G. (2008). In vitro cytogenetic results supporting a DNA nonreactive mechanism for ochratoxin A, potentially relevant for its carcinogenicity. Chem Res Toxicol 21:1235–1243.

Muñoz K, Blaszkewicz M, Degen G. (In press). Simultaneous analysis of ochratoxin A and its major metabolite ochratoxin alpha in plasma and urine for an advanced biomonitoring of the mycotoxin. J Chromatogr B 2010.878.2623–2629.

Muñoz K, Vega M, Rios G, Muñoz S, Madariaga R. (2006). Preliminary study of Ochratoxin A in human plasma in agricultural zones of Chile and its relation to food consumption. Food Chem Toxicol 44:1884–1889.

Navas SA, Sabino M, Rodriguez-Amaya DB. (2005). Aflatoxin M1 and ochratoxin A in a human milk bank in the city of São Paulo, Brazil. Food Addit Contam 22:457–462.

Nikolov IG, Chernozemsky IN, Petkova-Bocharova T, Vrabcheva T, Stojchev I, Bankov B, Castegnaro M, Pfohl-Leszkowicz A, Dragacci S, Day N, Gill C, Useleber E, Dietrich R, Martlbauer E. (2002). Review of the cooperative studies of the national oncological centre in Bulgaria on Balkan endemic nephropathy and associated urinary tract tumours (1991–2001). Facta Univ Ser Med Biol 9:119–122.

Nordic Working Group on Food Toxicology and Risk Evaluation. (1991). Health Evaluation of ochratoxin A in food products. Nordic Seminar and Work Report 545. Copenhagen, Denmark: Nordic Council of Ministers, 1–26.

Obrecht-Pflumio S, Dirheimer G. (2000). In vitro DNA and dGMP adducts formation caused by ochratoxin A. Chem Biol Interact 127:29–44.

Ostry V, Malir F, Roubal T, Skarkova J, Ruprich J, Cerna M, Creppy EE. (2005). Monitoring of mycotoxin biomarkers in the Czech Republic. Mycot Res 21:49–52.

Özçelik N, Koşar A, Soysal D. (2001). Ochratoxin A in human serum samples collected in Isparta-Turkey from healthy individuals and individuals suffering from different urinary disorders. Toxicol Lett 121:9–13.

Pacin AM, Ciancio Bovier EV, Motta E, Resnik SL, Villa D, Olsen M. (2008). Survey of Argentinean human plasma for ochratoxin A. Food Addit Contam Part A 25:635–641.

Palli D, Miraglia M, Saieva C, Masala G, Cava E, Colatosti M, Corsi AM, Russo A, Brera C. (1999). Serum levels of Ochratoxin A in healthy adults in Tuscany: Correlation with individual characteristics and between repeat measurements. Cancer Epidemiol Biomark Prevent 8:265–269.

Pascale M, Visconti A. (2000). Rapid method for the determination of ochratoxin A in urine by immunoaffinity column clean-up and high-performance liquid chromatography. Mycopathology 152:91–95.

Pena A, Seifrtová M, Lino CM, Silveira MIN, Solich P. (2006). Estimation of ochratoxin A in Portuguese population: New data on the occurrence in human urine by high performance liquid chromatography with fluorescence detection. Food Chem Toxicol 4:1449–1454.

Peraica M, Domijan A-M, Fuchs R, Lucić A, Radić B. (1999). The occurrence of ochratoxin A in blood in general population of Croatia. Toxicol Lett 110:105–112.

Peraica M, Domijan AM, Matasin M, Lucić A, Radić B, Delas F, Horvat M, Bosanac I, Balija M, Grgičević D. (2001). Variations of ochratoxin A concentrations in the blood of healthy populations in some Croatian cities. Arch Toxicol 75:410–414.

Petkova-Bocharova T, Castegnaro M. (1985). Ochratoxin A contamination of cereal in an area of high incidence of Balkan endemic nephropathy in Bulgaria. Food Addit Contam 2:267–270.

Petkova-Bocharova T, Castegnaro M, Pfohl-Leszkowicz A, Garren L, Grosso F, Nikolov I, Vrabcheva T, Dragacci S, Chernozemsky IN. (2003). Analysis of ochratoxin A in serum and urine of inhabitants from an area with Balkan endemic nephropathy: A one month follow up study. Facta Univ Ser Med Biol 10:62–68.

Petkova-Bocharova T, Chernozemsky IN, Castegnaro M. (1988). Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria. Food Addit Contam 5:299–301.

Pfohl-Leszkowicz A, Grosse Y, Kane A, Creppy EE, Dirheimer G. (1993). Differential DNA adduct formation and disappearance in the mouse tissues after treatment with the mycotoxin ochratoxin A. Mutat Res 289:265–273.

Pfohl-Leszkowicz A. (2009). Ochratoxin A and aristolochic acid involvement in nephropathies and associated urothelial tract tumours. Arh Hig Rada Toksikol 60:465–483.

Pfohl-Leszkowicz A, Manderville RA. (2007). Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res 51:61–99.

Pfohl-Leszkowicz A, Petkova-Bocharova T, Chernozemsky IN, Castegnaro M. (2002). Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins. Food Addit Contam 19:282–302.

Pfohl-Leszkowicz A, Pinelli E, Bartsch H, Mohr U, Castegnaro M. (1998). Sex- and strain-specific expression of cytochrome P450s in ochratoxin A-induced genotoxicity and carcinogenicity in rats. Mol carcinog 23:76–85.

Pfohl-Leszkowicz A, Tozlovanu M, Manderville R, Peraica M, Castegnaro M, Stefanovic V. (2007). New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor. Mol Nutr Food Res 51:1131–1146.

Poirier MC, Santella RM, Weston A. (2000). Carcinogen macromolecular adducts and their measurement. Carcinogenesis 21:353–359.

Postupolski J, Karłowski K, Kubik P. (2006). Ochratoxin a in maternal and foetal blood and in maternal milk. Rocz Panstw Zakl Hig 57:23–30.

Radić B, Fuchs R, Peraica M, Lucić A. (1997). Ochratoxin A in human sera in the area with endemic nephropathy in Croatia. Toxicol Lett 91:105–109.

Rizzo A, Eskola M, Atroshi F. (2002). Ochratoxin A in cereals, foodstuffs and human plasma. Eur J Plant Pathol 108:631–637.

Roth A, Chakor K, Creepy EE, Kane A, Roschenthaler R, Dirheimer G. (1988). Evidence for an enterohepatic circulation of ochratoxin A in mice. Toxicology 48:293–308.

Sabolić I, Asif AR, Budach WE, Wanke C, Bahn A, Burckhardt G. (2007). Gender differences in kidney function. Pflugers Arch Eur J Physiol 455: 397–429.

Sangare-Tigori B, Moukha S, Kouadio JH, Dano DS, Betbeder A-M, Achour A, Creppy EE. (2006). Ochratoxin A in human blood in Abidjan, Côte d'Ivoire. Toxicon 47:894–900.

Sauvant C, Holzinger H, Gekle M. (2005). Proximal tubular toxicity of ochratoxin A is amplified by simultaneous inhibition of the extracellular signal-regulated kinases 1/2. J Pharmacol Exp Ther 313:234–241.

SCF-Scientific Committee on Food. (1998). EC report, Opinion on ochratoxin A, expressed on 17 September 1998. Health and Consumers Directorate-General. Brussels, Belgium.

Schlatter C, Studer RJ, Rasonyi T. (1996). Carcinogenicity and kinetic aspects of ochratoxin A. Food Addit Contam 43–44.

Schwartz GG. (2002). Hypothesis: Does ochratoxin A cause testicular cancer? Cancer Causes Cont 13:91–100.

Schwerdt G, Freudinger R, Silbernagl S, Gekle M. (1999). Ochratoxin A-binding proteins in rat organs and plasma and different cell lines of the kidney. Toxicology 135:1–10.

Schwerdt G, Holzinger H, Königs M, Humpf H-U, Gekle M. (2009). Effect of ochratoxin A on cell survival and collagen homeostasis in human mesangial cells in primary culture. Food Chem Toxicol 47:209–213.

Scott PM. (2005). Biomarkers of human exposure to ochratoxin A. Food Addit Contam 22(Supp 1):99–107.

Shephard GS, Van der Westhuizen L, Sewram V. (2007). Biomarkers of exposure to fumonisin mycotoxins: A review. Food Addit Contam 24:1196–1201.

Skaug MA, Helland I, Solvoll K, Saugstad OD. (2001). Presence of ochratoxin A in human milk in relation to dietary intake. Food Addit Contam 18:321–327.

Skaug MA, Størmer FC, Saugstad OD. (1998). Ochratoxin A: A naturally occurring mycotoxin found in human milk samples from Norway. Acta Pædiatr 87:1275–1278.

Solti L, Pécsi T, Barna-Vetró I, Szász F, Biró K, Szabó E. (1999). Analysis of serum and seminal plasma after feeding ochratoxin A with breeding boars. Anim Reprod Sci 56:123–132.

Stefanovic V, Cukuranovic R, Mitic-Zlatkovic M, Hall PW. (2002). Increased urinary albumin excretion in children from families with Balkan nephropathy. Pediatr Nephrol 17:913–916.

Stefanovic V, Mitic-Zlatkovic M, Cukuranovic R, Miljkovic P, Pavlovic NM, Vlahovic P. (1991). β2-microglobulin in patients with Balkan nephropathy and in healthy members of their families. Kidney Int 40:21–26.

Stefanovic V, Mitic-Zlatkovic M, Cukuranovic R, Vlahovic P. (2003). Increased urinary protein excretion in children from families with Balkan endemic nephropathy. Nephron Clin Pract 95:116–120.

Stoev SD. (2010). Studies on carcinogenic and toxic effects of ochratoxin A in chicks. Toxins 2:649–664.

Stoev SD, Vitanov S, Anguelov G, Petkova-Bocharova T, Creppy EE. (2001). Experimental mycotoxic nephropathy in pigs provoked by a diet containing ochratoxin A and penicillic acid. Vet Res Com 25:205–223.

Stojkovic R, Hult S, Gamulin R, Plestina R. (1984). High affinity binding of ochratoxin A to plasma constituents. Biochem Int 9:33–38.

Studer-Rohr I, Schlatter J, Dietrich DR. (2000). Kinetic parameters and intraindividual fluctuations of ochratoxin A plasma levels in humans. Arch Toxicol 74:499–510.

Suzuki S, Satoh T, Yamazaki M. (1975). Effect of ochratoxin A on carbohydrate metabolism in rat liver. Toxicol Appl Pharmacol 32:116–122.

Thuvander A, Paulsen JE, Axberg K, Johansson N, Vidnes A, Enghardt-Barbieri H, Trygg K, Lund-Larsen K, Jahrl S, Widenfalk A, Bosnes V, Alexander J, Hult K, Olsen M. (2001). Levels of ochratoxin A in blood from Norwegian and Swedish blood donors and their possible correlation with food consumption. Food Chem Toxicol 39:1145–1151.

Timbrell JA. (1998). Biomarkers in toxicology. Toxicology 129:1–12.

Tozlovanu M, Faucet-Marquis V, Pfohl-Leszkowicz A, Manderville RA (2006). Genotoxicity of the hydroquinone metabolite of ochratoxin A: Structure-activity relationships for covalent DNA adduction. Chem Res Toxicol 19:1241–1247.

Turconi G, Guarcello M, Livieri C, Comizzoli S, Maccarini L, Castellazzi AM, Pietri A, Piva G, Roggi C. (2004). Evaluation of xenobiotics in human milk and ingestion by the newborn—An epidemiological survey in Lombardy (Northern Italy). Eur J Nutr 43:191–197.

Ueno Y, Maki S, Lin J, Furuya M, Sugiura Y, Kawamura O. (1998). A 4-year study of plasma ochratoxin a in a selected population in Tokyo by immunoassay and immunoaffinity column-linked HPLC. Food Chem Toxicol 36:445–449.

Vaidya VS, Ferguson MA, Bonventre JV. (2008). Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol 48:463–493.

Valenta H. (1998). Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. J Chromatogr A 815:75–92.

Van der Merwe KJ, Steyn PS, Fourie L, Scott DB, Theron JJ. (1965). Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature 205:1112–1113.

Vatinno R, Aresta A, Zambonin CG, Palmisano F. (2007). Determination of ochratoxin A in human urine by solid-phase microextraction coupled with liquid chromatography-fluorescence detection. J Pharm Biomed Anal 44:1014–1018.

Wafa EW, Yahya RS, Sobh MA, Eraky I, El-Baz M, El-Gayar HAM, Betbeder AM, Creppy EE. (1998). Human ochratoxicosis and nephropathy in Egypt: A preliminary study. Hum Exp Toxicol 17:124–129.

Zimmerli B, Dick R. (1995). Determination of ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: Methodology and Swiss data. J Chromatogr B Biomed Appl 666:85–99.